T H E S I S

presented for the

Degree of Doctor of Philosophy

University of Edinburgh

on

ZONE LINES IN PLANT TISSUES

by

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June 1933.

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Foreword.

THE writer feels that the most convenient and concise way of presenting the material facts of this research on the zone lines in plants is in the form of the published paper. Accordingly, this thesis consists of two papers, one dealing with the zone lines of Xylaria polymorpha and the other with those of Armillaria mellea, each of which is complete in itself. The papers, however, are bound together by the interpretation of the zone line of Xylaria and Armillaria as the bounding layer of a sclerotium-like body. Further, the development of this idea can be traced from the original description of the entostroma in the Xylaria paper to the later recognition of the pseudosclerotium in the case of Armillaria mellea.

[FROM THE ANNALS OF APPLIED BIOLOGY, VOL. XX, No. 1,
pp. 123–145, FEBRUARY, 1933.]

ZONE LINES IN PLANT TISSUES

I. THE BLACK LINES FORMED BY *XYLARIA POLYMORPHA* (PERS.) GREV. IN HARDWOODS

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(With Plates XI–XIII.)

CONTENTS.

I. Introduction.

The zone lines formed by certain fungi in the invaded host tissues have presented a puzzling problem to mycologists. Their structure, formation and significance are little understood, while the accounts in the literature are generally obscure or speculative. Hubert (13) has pointed out the diagnostic value of zone lines, particularly those associated with incipient decay, but until we have more accurate knowledge about them, additional evidence is necessary in the identification of a rot. The confusion existing in the literature may be partly explained by the nature of the problem, since the zone lines may be formed by a number of different fungi in a variety of different ways.

White (30), referring to black lines in general, states that cultural or other evidence shows that when they do occur more than one species of fungus is at work. He says that black lines are well known to pathologists and are produced at the point of contact of two invading fungi in the case of many pairs of wood-destroying fungi. Weir (29) has stated that "practically in every case where two distinct wood-destroying fungi occupy the same substratum, the mycelium or the wood decayed by each is sharply outlined or bounded by infiltrated zones of thylose-like deposits in the cells. These zones or layers are similar to the lines of demarcation often appearing in the rot of a single species and which separate the diseased from the healthy wood. The antagonism of the mycelium of one species to that of another is a marked characteristic of many wood-rotting fungi. The rot of *Fomes pinicola* and that of *Fomes fomentarius* in the wood of the same birch tree is always sharply separated by a conspicuous black line although the decay produced by each species is quite characteristic. The same evident antagonism is often true in the case of purely saprophytic species when occupying the same substratum." Hubert (13) has made some observations on such zone lines and states that they occur between the rots of *Polyporus anceps* and *Lenzites sepiaria* in *Picea canadensis* and *Fomes applanatus* and *Stereum frustulosum* in *Quercus* sp. A double zone line may often be formed. No satisfactory account exists of the formation and microscopic structure of these zone lines formed between the mycelia of two different fungi on the same substratum, and the record of their occurrence depends chiefly upon the identification of the invading fungi from sporophores or the presence of typical rots.

Hartig (11) has described the formation of a black line in wood by the attack of a single fungus, *Armillaria mellea*. According to Hiley (12) the honey fungus comes to occupy the cambium and from this position invades the wood on one side and the bark on the other. The extent of the invasion in the xylem is marked by a black line, one or more tracheides thick, and generally in the shape of a triangle with its base in the cambium. This black line consists of densely packed, bladder-shaped hyphae in the lumina of the tracheides and fibres. It has been stated by Hartig that the black line accompanies the advance of the rot and that the wood after its passage gives a cellulose reaction and speedily dissolves from the lumen outwards. Hiley has illustrated the method of movement of the line and has stated that the bladder hyphae are a more active stage of the fungus whereby the wood tissues are broken down much more readily. According to Butler (4) the black lines of *Armillaria mellea* seem to be laid down in the position which they continue to occupy permanently. The whole process, he declared, is much more like the development of some defensive reaction on the part of the host or the parasite. Brooks (2), referring to a disease of rubber caused by *Ustulina zonata*, stated that "these conspicuous black lines are caused by the fungus forming a kind of sclerotic plate in the tissues, the difference between this and the typical sclerotium being that the latter does not include within it portions of the tissue of the host. These black zones differ from rhizomorphic strands in the same respect." Brooks, however, figures the black line as a black mass with no apparent structure, and his suggestion as to the nature of these black lines was not founded upon experimental evidence. *Nummularia pithodes* and a "*Xylaria* species" have been observed by the same author to cause similar black lines in wood. Sharples (25) describes the black line formed by *Ustulina zonata* in rubber as formed by the aggregation and massing of hyaline hyphae and later the deposition in the cells of a carbonaceous material. Panisset (16) has described *Daldinia concentrica* as causing black zones in ash. She states that the significance of the black dichotomous hyphae, which are the principal constituents of the black zones, is unknown, but suggests that they may be compared with the brown, bladder hyphae of the *Armillaria* black line since both kinds lose their protoplasmic contents. Another suggestion is that the black zones represent an arrested development of ascocarps.

Rhoads (21), in a paper entitled "The black zones formed by wood-destroying fungi," has dealt with black lines in a general way and was apparently under the impression that the structure of the black lines formed by the particular fungus he worked with was typical of the black lines formed by the other fungi. He dealt with the black lines caused by *Coriolus prolificans* (*Polystictus versicolor*) on *Hicoria minima*, and stated that the black zones in wood caused by fungi are in substance the same as those caused by wounds under natural conditions, being composed of natural decomposition products which infiltrate the cells and form droplets in the lumina. The continual occurrence of blackish zones between decayed and undecayed wood is due to the fact that the decomposition products are destroyed with the wood while new ones are formed from the wood as fast as it is attacked by the advancing fungus. The formation of the brown decomposition products depends upon (*a*) the presence of dead cells, (*b*) an optimum supply of moisture, and (*c*) sufficient oxygen to cause oxidation. He further stated that "the decomposition products whose formation is due to the action of wood-destroying fungi have proven to be a group of substances analogous to or nearly identical with the decomposition products, which arise under certain circumstances in dead wood that is entirely free from fungal attack and which have been known under the name of 'wound gum.' Their formation is greatly accelerated by the presence of wood-destroying fungi which greatly hasten the decomposition and hence the oxidation." There seems to be no doubt that "wound gum" is capable of forming black lines, but it is by no means the only cause, as Rhoads has suggested.

Hubert (13) has indicated that zone lines occur more commonly in pieces of infected wood most subject to desiccation. Hartig (11) states that the zone lines in oak wood infected with *Fomes igniarius* are due primarily to the entry of air into infected wood tissues. Lindroth (14) figures a zone line formation just behind the freshly cut surfaces of birch wood infected with *Polyporus nigricans*. Hubert (13) suggests that, "Desiccation and oxidation of the decomposition by-products seems responsible for the formation of these lines." Data on the appearance of zone lines in wood inoculated with pure cultures of wood-destroying fungi support this view. The zone lines invariably appeared during the periods when the moisture in the tubes and in the inoculated blocks had nearly evaporated. Zone lines were secured in the following cultures "on the hosts named; *Trametes pini* in *Picea sitchensis* heartwood, *Fomes igniarius* in *Populus tremuloides* sapwood, *Xylaria polymorpha* in *Tilia americana* heartwood, *Polyporus adustus* (?) in *Populus tremuloides* sapwood, *Hymenochaete rubiginosa* in *Populus tremuloides* heartwood, *Fomes applanatus* in *Populus tremuloides* sapwood, *Ganoderma curtisii* in *Populus tremuloides* sapwood and in pure cultures on malt agar, the mycelium extending to the cotton plug and forming a zone line across the plug." He continued that the data indicated that whenever freshly cut pieces of wood infected with certain fungi are placed in a dry room or left to dry in the open, characteristic zone lines are formed a short distance back from and parallel to the cut surfaces. Field observations often show zone lines in the upper portions of rotted stumps in the region where excessive drying occurred, roughly parallel to the cut surfaces from which evaporation took place. It seems probable that desiccation is the cause of a particular kind of zone line, but nothing is known of the microscopic structure of the lines, which Hubert following Rhoads believed to be produced by the oxidation of decomposition products similar in structure to "wound gum." It is not proposed to deal here with the extensive literature on "wound gum," but mention must be made of the more recent work of Brooks and Storey (3) and Swarbrick (26) who maintain that the production of "wound gum" is the reaction of living tissues to invasion as against the decomposition origin postulated by Rhoads.

It seems, then, that in the present state of our knowledge, we must recognise the existence of zone lines in wood due to three distinct agencies. These are (1) the antagonism of the mycelia of two different fungi occupying the same substratum, *e.g.* between the rots produced by *Fomes* *pinicola* and *F. fomentarius* in birch (29); (2) the action of the single mycelium of certain fungi, such as *Armillaria*, *Daldinia*, *Ustulina*, etc.; and (3) the production of "wound gum" stimulated by (*a*) natural wounding, (*b*) parasitic invasion, fungal or bacterial, or (*c*) desiccation (13). It will readily be seen that there are no easy criteria for judging the origin of zone lines macroscopically in wood. The author has collected and had presented to him a considerable number of specimens of wood rots containing zone lines which, from preliminary examination, appear to fit into the groups set out above. Before any comparative study of these lines for use in the diagnosis of decay can be attempted, it will be necessary to supply an adequate descriptive and anatomical account supported by cultural and experimental evidence of the typical members of each group. It is the object, then, of this paper to provide a description of the black lines formed by *Xylaria polymorpha* in hardwoods together with an account of their origin, formation and biological significance.

II. *XYLARIA POLYMORPHA* (Pers.) Grev.

Xylaria polymorpha is one of the commonest pyrenomycetes found on decaying stumps of deciduous trees. Ramsbottom (20) lists this fungus as "common," and it is certainly of quite frequent occurrence on wood of *Fagus*, *Acer*, *Quercus*, *Fraxinus* and other hardwoods. According to Saccardo (23) *Xylaria polymorpha* has been collected by Mougeot on the decaying wood of *Pinus sylvestris*, while Therry has collected it on the stem of *Araucaria*.

The author had already in his possession several specimens of beech showing very irregular black lines believed to be caused by *Xylaria* and further obtained at the Royal Botanic Garden, Edinburgh, a stump of *Acer pseudoplatanus* bearing a luxuriant growth of *Xylaria polymorpha* fructifications (Plate XII, fig. 1). Sections cut from the stump showed, on examination, the presence of black lines in the wood. The fungal fructifications were identified as *X. polymorpha* (Pers.) Grev. Saccardo (24) describes five varieties of this fungus based chiefly on shape. In Lloyd's (15) opinion they are not worth recording because *X. polymorpha*, as its name implies, is exceedingly irregular in shape. The temperature variations and other climatic factors of the environment determine to a much greater extent the shape of the fungus than does the nature of the matrix. There are, however, the critical characters of the species with which it agrees. These are a solid, white stroma, black on the outside; a wrinkled surface due to contraction; perithecia that do not protrude and an ascospore length from 24 to 32 μ. The exact ascospore size of this material was $25 \times 7\,\mu$.

Single ascospore cultures were obtained from the fructifications on the *Acer* stump, while isolations were made from the black lines in the wood by cutting out in a sterile manner pieces of black line and transferring them to malt agar plates. The resulting pure cultures agreed in every respect. A number of isolations was made from the black lines in the beech wood and more ascospore cultures were obtained from batches of *X. polymorpha* from different localities. The cultures from these various sources were all closely compared and were found to be identical in their cultural characteristics.

III. Cultural characteristics.

The ascospores of *X. polymorpha* germinated readily by the brown outer membrane splitting down one side, as described by Vincens (28), to allow the emergence of the hyaline germ tube which gave rise to the mycelium. The colonies of *X. polymorpha* have appressed mycelium with indistinct zonation. The margins of the colonies are generally lobed, the lobes being separated by radial ridges which arise from the centre of the colony (Plate XII, fig. 2). On sterilised wood blocks the mycelium is at first very delicate with little zonation, the size and other features varying with the wood employed. Later, the whole block of wood becomes covered with a thick white mycelium, which eventually becomes quite black. Luxuriant vegetative growth with pronounced zonation is obtained when the fungus is grown upon sterile carrot slabs in the daylight (Plate XII, fig. 3). The zones apparently correspond to the alternate day and night periods, which form in turn bands of appressed and aerial hyphae. On artificial media the colonies are at first pure white in colour, but later, with the formation of dark hyphae below the white mycelial mat, they become neutral grey. When grown on oat agar the black incrustation of the dark hyphae appears after about a week, while at the end of 10 days erect stromata begin to form in the centre and at the margin of the colony (Plate XII, fig. 2). After a month's incubation at 25° C., when grown in tubes or a longer period in plates, the stromata reach 2 or 3 in. in height and about $\frac{3}{16}$ in. in diameter and are covered at the tips with a coating of light brown conidia.

The fungus has been cultured on a number of artificial media including the following: oat agar, Etter's (6) medium, *C.*[1] medium, maize extract

[1] This medium is made up by mixing dry 35 gm. of beech sawdust with 100 gm. each of Quaker oats and maize meal. Fill the culture flasks to a depth of 2 in. and then saturate with a malt extract solution of 15 gm. malt extract in 1000 c.c. Sterilise.

agar, malt extract agar, potato dextrose agar, Czapek's solution agar, Dox agar, soil extract agar, starch agar, agar and gelatine. On the following sterilised plant parts, carrot, potato, and potato with the addition of 1 per cent. glycerine, excellent growth was obtained. The mycelium grew well on beech, *Acer*, ash, elm, oak, lime, and poplar blocks in large tubes ($8 \times 1\frac{1}{2}$ in.) sterilised by two exposures of 30 min. at 25 lb. steam pressure and on unsterilised beech blocks. Excellent mycelial growth and rapid stromatal production were obtained with oat agar, and Etter's medium. The growth on the modified Etter's medium was quite remarkable, stromata 6 in. high and some $\frac{1}{4}$ in. in diameter being produced. Malt extract agar, carrot, potato, potato with 1 per cent. glycerine and potato dextrose agar gave good growths while starch agar, Czapek's solution agar, Dox agar and soil extract agar gave much poorer results. According to Freeman (7) and Bronsart (1) *X. Hypoxylon* requires asparagin as a nitrogen source in the formation of stromata, while *X. polymorpha* requires ammonium nitrate. The addition of varying quantities from a trace to 1 per cent. of asparagin and ammonium nitrate to standard and synthetic media has failed to stimulate stromatal production in excess of the normal. On gelatine the fungus grew strongly showing an infundibulum liquefaction within a few days and finally complete liquefaction of the gelatine.

A number of isolations from wood while showing the characteristic *Xylaria* mycelium have remained sterile, producing no stromata. Guéguen (10) first noted this phenomenon and suggested that the development of *X. polymorpha* in culture was correlated with its seasonable development. He found that February to March was the best period for inoculation, and that cultures made before then tended to be sterile. Wolf and Cromwell (31) and Fromme and Thomas (9) have also observed that cultures may remain sterile for a considerable time before fructifying. According to the author's experiments the time of inoculation does not seem to have any effect in the production of stromata.

It was found that the most convenient temperature for growing cultures was 25° C. with the optimum about 27° C. Wolf and Cromwell (31) investigating a *Xylaria* root rot of apple found that the most luxuriant growth occurred in cultures kept in an ice-box at 11–13° C. In the case of *X. polymorpha* mycelial growth falls off below 16° C. and is very poor at 10–12° C.

When cultures are sealed up with a pyrogallic acid and caustic soda mixture to produce anaerobiosis, the growth continues slowly for a few days and then stops. The mycelium is eventually killed. If the cultures are grown in sealed bottles with confined air, the mycelium remains white and in place of the stromata white sterile humps are formed. These humps are seldom more than $\frac{1}{2}$ in. high.

IV. Development of the fungus in culture.

The young filaments produced by the germinating ascospore are hyaline, branched and densely granular, with an average diameter of $1–2\,\mu$. Later, the hyphae lose their granular contents and become more closely septate and brown in colour. The transition from hyaline to brown hyphae in culture is a gradual process with a number of easily recognisable intermediary stages. The first stage is seen in the enlargement of the hyaline hyphae from a diameter of about $1{\cdot}5–3\,\mu$ and the appearance of a greater number of vacuoles in the protoplasm. Later, the walls thicken and closely placed septa make their appearance (Plate XIII, fig. 4). The hyphae are now a uniform brown colour due to the deposition on the walls of a brown substance. About this stage the granular protoplasm finally disappears and its place is taken by clear contents. The long, straight hyphae with the well-defined septa begin to darken in colour, while the single cell units swell up and become bladder-shaped with a diameter from 4 to $10\,\mu$. These bladder cells which are now dark brown in colour tend to aggregate into black masses in which it is exceedingly difficult to differentiate the unit cells. This is practically the condition that is found on the black line in wood.

Eventually stromata appear in the cultures (Plate XII, figs. 2 and 5). When grown on *C.* medium these may attain a height of 6 in. with a $\frac{1}{4}$ in. diameter and are generally quite cylindrical and unbranched. The stromata present an odd appearance, having large globules of exuded water adhering to the surface, and differ considerably in appearance from those of *X. polymorpha* in nature. Cultures grown upon oat agar, with the addition of beech-wood sawdust, in large dishes have shown after 3 months' incubation at 25° C. stromata about $\frac{3}{4}$ in. high and exactly like miniature *X. polymorpha* fructifications as they appear in nature. In addition sterile coremia as have been described by Guéguen (10) were prominent on this medium. When cultures were grown on oat agar in Erlenmeyer flasks at 25° C. until the stromata were well developed and then transferred to a cooler incubator at 17° C. the stromata became covered with brown hairs about 3 mm. long which projected at right angles to the stroma and extended from the base as far as the conidial bearing region at the tip. A single hair generally consists of a number of long, straight, unbranched hyphae, which are distinctly septate and brown in colour, arranged parallel to each other and closely adhering to form a single unit. The presence of these hairs gives the stroma a very curious appearance (Plate XII, fig. 7). Another feature which results from the exposure to slightly lower temperature is the branching of the stroma. Generally when grown throughout at 25° C. the stromata remain straight and unbranched; if, however, the culture is exposed to a temperature of 17° C. for 24 hours the growing point at the tip appears to be checked and there is no further elongation of the stroma. Very soon, however, a whorl of new shoots, from two to four in number, makes its appearance from just below the checked tip and in this way the growth of the stroma is continued. This process of checking the tips can be carried out a number of times to produce stromata bearing successive whorls of branches. It might be suggested, since the shape and appearance of the stroma in culture can be varied so much, that the great irregularity in shape in the genus *Xylaria* and in particular in the species *polymorpha* may be due to the occurrence of this phenomenon in nature.

The conidia, which appear as a brown coating at the tip of the stroma, are hyaline and elongated oval in outline with a blunt truncate pedicel. They measure $10 \times 3{\cdot}5\,\mu$ and are borne on palisade-like conidiophores. The conidia germinate readily to produce a single germ tube which gives rise to the mycelium, and in this way seven successive generations of *X. polymorpha* have been raised in culture.

A number of media have been experimented with, but so far all attempts to obtain perithecia and ascospores in culture have been unsuccessful. Complete stromata have been sectioned, but no sign of perithecial formation has been detected. With the exception of Freeman (7), who states that ascospores were produced in cultures of *X. Hypoxylon* on steamed blocks of elm wood in 6 or 8 weeks, ascospores are unrecorded in culture for *X. Hypoxylon* and *X. polymorpha*.

V. BLACK LINES PRODUCED IN INOCULATED WOOD.

A number of wood-block cultures were set up to test the production by *Xylaria* of stromata and black lines on a wood substratum. The woods used were beech, *Acer*, lime, elm, ash, oak and poplar and at first young branches about ¾ in. in diameter and cut into 2-in. lengths were used as the test-pieces. Later, it was found that the hyphae penetrated the wood much more readily if blocks were used which had been cut as ½ in. thick transverse slices of the trunk and then divided into pieces 3×1 in., as in these pieces the largest possible area of "end-wood" was available for the entry of the fungus. The blocks were soaked in water for 24 hours before placing them on a cotton-wool base in large tubes ($8 \times 1\frac{1}{2}$ in.), and varying quantities of pure water were added before the whole was sterilised at 25 lb. steam pressure for 30 min. The test-pieces were inoculated with conidia and mycelium from *X. polymorpha* cultures and from isolations from black lines in beech and *Acer*. The cultures were incubated at 25° C. for 12 months and then the blocks were examined for black-line production.

The test-blocks were covered with a dense, felty mycelium, brown-black in colour and forming a thick crust on the wood. About 80 per cent. of the cultures bore stromata and conidia, as is shown in Plate XII, fig. 7. When the mycelium was scraped off the blocks black lines and ovals were found which were identical in appearance with those in the *Acer* stump but not nearly so numerous. In most of the cultures the black lines were superficial and did not penetrate the wood more than a short distance. It was observed that the cultures with little surface mycelium generally produced quite a large number of black lines in the wood. The cultures which had an exceedingly thick crust of mycelium, due to a larger amount of water being present in the tubes when inoculated, yielded only a delicate black tracery at the surface of the wood, although the thinner hyaline hyphae were present in the wood in large numbers. It is suggested that insufficient aeration may be a limiting factor in the formation of the black lines, as it has been shown previously that cultures grown in confined air do not develop the black hyphae which compose the black line but only the thinner hyaline hyphae and remain quite white in colour. A very striking feature, however, was the presence of distinct black lines in the cotton-wool at the bottom of the tubes (Plate XII, fig. 6). These black lines could be dissected out with care and were found to consist of a shell, complete, except at the point where the black lines came into contact with the glass, which thus prevented their full development; so that the black circle seen in the cotton-wool is really a section through an egg-shaped body which has the black line for its circumference. The significance of these ovoid bodies will be dealt with in a later section.

Since cultures raised from single ascospores of the *X. polymorpha* fructifications on the *Acer* stump and from the isolations from black lines in the stump have been found to be identical in cultural characteristics and stromatal and conidial formation, there could be no doubt as to the identity of the fungus causing the black lines in the *Acer* stump. Isolations from black lines in beech, in which wood they are most commonly found, have yielded cultures identical with those of *X. polymorpha,* and the morphology of these black lines agrees in every particular with that of the *Acer* wood. In addition, conidia and mycelium of *X. polymorpha* have produced black lines in test-pieces of beech, *Acer*, lime, elm, ash, oak and poplar, as have also isolations from black lines in beech and *Acer*. It seems, however, that *X. polymorpha* is capable of producing black lines in a number of hardwoods just as its allied genera, *Ustulina* (2) and *Daldinia* (16), have already been shown to produce black lines in rubber and ash respectively.

VI. MORPHOLOGY OF THE BLACK LINE.

Hubert (13) has figured black lines in the trunk of *Acer rubrum* produced by *X. polymorpha*. According to him the zone lines are formed round areas which originate from the various foci of infection and produce double lines where such areas meet. Petch (17, 18, 19), investigating a root disease of rubber and tea caused by *X. Thwaitesii* Cooke, has observed that black lines and ovals may be present and that the hardness of the wood, despite its intense discoloration, was a striking feature. Fromme (8) stated that black lines or blocking layers are sometimes seen in the wood in the case of apple root rot caused by *Xylaria* spp. Brooks (2), investigating a disease of rubber caused by *Ustulina zonata*, described conspicuous black lines about $\frac{1}{32}$ in. thick occurring in the wood. The same author has found *Nummularia pithodes* (B. and Br.) Petch and a species of *Xylaria* (probably *X. Thwaitesii*) causing similar black lines in the wood of rubber.

The author had the opportunity recently of examining a complete 60 ft. specimen of *Fagus sylvatica* which had been apparently killed by *Fomes applanatus*. At the base of the trunk, some 2 ft. 6 in. in diameter, the black lines of *X. polymorpha* were extremely numerous although no fructifications were present. The black lines extend in what appear to be vertical plates which may fuse frequently in the ascent of the trunk. These plates when seen in section appear as thick lines, hair lines, double lines, ovals or "islands," all branching and then anastomosing to form crazy patterns in the wood. Plate XI, fig. 1, shows part of an oblique-transverse section 4 ft. from the base and shows the black lines distinctly. Apparently no attempt is made by the fungus to follow the easiest path through the wood, as the black lines pass through all types of tissue and penetrate both bark and sapwood as can be seen in this photograph. A remarkable feature is the soundness of the wood, which is extremely hard, especially at the black line, of normal colour and free from brashness. Plate XI, fig. 2, shows a transverse section of one of the twin trunks cut 7 ft. above the ground. "Islands" and ovals are especially prominent in this section, which may be explained by the fact that the black lines, as they appear in section, are actually the circumferences of a number of ovoid or narrow ellipsoid bodies whose long axes are parallel to the tree axis. Thus these "islands" represent sections cut through these ovoid bodies close to the apices. The actual number of black lines present in this top section is much smaller than at the base, so that it can be concluded that the furthermost extent of the black lines has almost been reached. Further sections confirm this, as the black plates converge to their apices and finally disappear 9 ft. from the base.

When the invaded wood tissues are sectioned and stained with safranin and picro-aniline blue (5) a number of minute hyphae stain up bright blue around the black line. The black line itself consists of a dense mass of brown bladder cells occupying the lumina of the vessels, fibres and cells of the medullary ray in the path of the line. The black line is best examined in unstained longitudinal radial sections (Plate XIII, fig. 3) which must be as thin as possible so that in microtoming longitudinal sections are preferred to transverse ones. Examination of many of the black lines produced in culture blocks has shown a number of stages in the formation of the black line. It appears that first of all the position which the black line is to occupy becomes marked out by the aggregation of thin, hyaline hyphae. Later, the hyaline hyphae swell up and become closely septate forming the brown bladder cells as in culture. The bladder hyphae first make their appearance in rows of isolated patches of irregular shape, then gradually the intervening spaces become filled with the brown hyphae and the line begins to assume a distinct and clear-cut appearance with the close packing of the hyphae in every available cavity within the limits of the black line. Some of the bladder cells then collapse and their contents stain the walls of the vessels, penetrate the pits between the cells of the medullary ray and fill the interstices between the other bladder cells. The result is to produce in a complete section the effect of a closely packed barrier through which penetration must be nearly impossible. In addition, this packing of the swollen hyphae lends mechanical support and rigidity to the black line which is always very much harder than the surrounding wood. Indeed, so tightly are these swollen hyphae packed that it has been found possible in sufficiently decayed wood to dissect out the black lines in the form of sheets several square inches in area. These black plates consist only of the swollen hyphae and remnants of the walls of the cells in which the hyphae were formed.

The brown substance which stains the cell walls and fills the pits is of unknown composition, and very little is known about its formation. It was found to be insoluble in acetone, alcohol, benzol, carbon disulphide, chloroform, methyl ether and hot water. Concentrated sulphuric, hydrochloric and nitric acids and strong potassium, ammonium and sodium hydroxides, hot or cold caused no reaction. Iodine, however, gave a blue reaction along the black line when microsections were tested, and Eau de Javelle completely bleached the line after 24 hours.

Hiley has suggested that the bladder cells in a somewhat similar black line caused by *Armillaria mellea* are "a more active stage in the metabolism of the fungus" which breaks down the wood tissues more readily. It must be remarked, however, that if such is the function of the hyphae of the black line, they seem singularly ill-adapted to their function. It is to be expected that hyphae of high functional activity would present the maximum surface area for assimilation and also be well spaced to allow of sufficient aeration. This does not seem to be the case with the hyphae of the black line. In fact, the line hardly varies in breadth but remains clear-cut with no apparent tendency to spread. This point will be dealt with more fully in a subsequent communication on the black lines caused by *Armillaria mellea*, but meanwhile it will be sufficient to add that in the sounder wood as shown in Plate XI, fig. 1, tests reveal scarcely any delignification and certainly no perceptible difference in the delignification on either side of the black line. On the other hand wood in a more decayed condition as in Plate XI, fig. 2, shows what at first sight appears to be a different condition of affairs. Towards the left-hand corner of this specimen and in the centre can be seen areas which judged by the difference in their colour seem to be at different stages of decay. These areas are bounded by black lines, so that it might be thought, and not without some reason, that the black lines represented the lines of advance of the fungus from different foci of decay. In the centre of the specimen is a considerably rotted area which has been caused by the *Fomes*. On top of this heart rot the black lines are superimposed, making the whole appear, with its differences in light and shade, not unlike a patchwork cover. If it were postulated that these black lines occur in the position they do quite fortuitously, the apparent differences in decay on either side of the black line could be explained by reference to an anatomical feature of the black line. This feature is the closely packed bladder hyphae, by virtue of which the black lines form complete barriers, such as might quite easily prevent the further spread of fungi and bacteria. To reconstruct what happened inside this tree it is necessary to get a mental picture of the successive invaders. The initial *Fomes applanatus* rot gaining access at the base spread upwards killing the wood and rotting the heart area. The brown zone which marks the advance of the *F. applanatus* in living wood cannot be easily seen, as this specimen had been standing dead for some time and later saprophytic fungi have practically removed the brown zone. As one of the first of these saprophytic fungi came *Xylaria*, whose hyaline mycelium spread throughout the dead tissues without causing any marked change in their appearance. Eventually the *Xylaria* developed its black lines so that all subsequent invaders entering by the same route as the *Xylaria* tended to be isolated by the barriers presented by the black plates, and thus arose the areas with different degrees of decay separated by black lines. To confirm this hypothesis a number of cultures was made from the various rot areas and several unidentified fungi along with *Fomes applanatus* were isolated from the typical rot, while *Xylaria* was only obtained after careful isolation from the black lines. In many cases bacteria seemed to be by far the chief invaders. Bacterial counts by direct observation and by dilution of equal weights of rotted tissue and subsequent platings have established without a doubt that there may be a considerable difference in the number of bacteria in adjoining areas separated by a black line. So that the impermeable nature of the *Xylaria* black line is responsible for the differences in decay in adjoining areas by the limitation it imposes on the secondary fungi and bacteria, which so commonly invade the later rots of *Fomes applanatus*.

VII. Zone lines of superimposed rots.

Not a little of the confusion existing over the formation of zone lines by wood-rotting fungi becomes understandable by reference to the specimen shown in Plate XIII, fig. 5. This photograph shows part of a beech-block attacked by a typical heart rot of *Fomes applanatus*. The region marked 1 is apparently normal wood, in colour and texture, and as yet uninfected. On the right of it and marked 2 is a dark brown band similar in appearance to that which has been described by White (30) as marking the advance of a *F. applanatus* rot. Section 4 consists of unnaturally white, much decayed wood, which represents the typical rot stage of the *Fomes* attack. Throughout this area and closely appressed to the brown band occur thin black lines (3) which have been identified culturally and morphologically as *X. polymorpha*.

The microscopic examination discloses that section 1 of the wood is normal with no hyphae or tyloses. Section 2, as can be seen in Plate XIII, fig. 6, contains a few hyphae, but a more prominent feature is the presence of tyloses which completely plug the vessels, while the parenchymatous cells and tracheides alongside are filled with a dark brown substance known as "wound gum" (*w.g.*). The "wound gum" is also found filling up the spaces between the tyloses in the vessels and in a number of cases in the tyloses themselves. It is the "wound gum" which is responsible for the dark colour of this portion of the wood. The black line marked 3 is a normal *Xylaria* black line with the difference that in this case the line also contains tyloses which were formed first as a result of the *F. applanatus* attack and in consequence the bladder mycelium has been compelled to fill up the only remaining space in the vessels, which are more or less isolated pockets between the tyloses and in the lumina of the tracheides and parenchymatous cells. In spite of this, however, the line loses none of its distinctness, while every available space within the limit of the line is packed with hyphae and stained with the brown pigment which always accompanies the *Xylaria* bladder hyphae. This pigment is quite distinct and must not be confused with the "wound gum." Both, in this case, occur within the black line; the "wound gum" occupying the lumina of the tyloses is characterised by its granular and fractured appearance in mounted sections while the *Xylaria* brown pigment is always transparent and does not seem to fracture. In section 4 the wood is considerably delignified, and the hyphae of later saprophytic fungi and bacteria are present in large numbers. The "wound gum" has disappeared, having been apparently bleached or absorbed by the hyphae, and while the tyloses remain for some little distance from the black line they, too, are eventually broken down and absorbed by the invading fungi and bacteria.

The various sections of the wood are sharply delimited and of extraordinarily different appearance. The specific gravities of the sections 1, 2 and 4 were determined by a volumeter method of water displacement and were found to be of the following approximate values:

S.G. sound wood, section 1	0·72
S.G. infiltrated wood, section 2 ...	0·89
S.G. decayed wood, section 4	0·48

It will be seen from these figures that the brown zone is considerably heavier than normal wood, and that the decayed wood is very much lighter than normal.

White (30), in his study of *F. applanatus*, refers to the brownish discoloured zone as marking the extreme limit of the advance of the fungus and making the line of demarcation from the sound and yet uninfected wood very distinct. The brown coloration is due to the "wound gum" which is found in the lumina of the parenchymatous cells, in the vessels and impregnating the walls of the tyloses. As neither the "wound gum" nor the tyloses occur in the sound tissues beyond the brown band, they must be regarded as a pathological condition and a phenomenon which is never found in sound wood. There is an extensive literature on the subject of "wound gum" but, briefly, there are two divergent theories at present in existence. One opinion maintains that "wound gum" originates after the death of the cells as an oxidation product, while the other declares it to be a vital reaction and a secretion from living protoplasm. The tyloses, however, cannot enter this discussion as they are only produced by living cells. But at the moment the evidence is in favour of both "wound gum" and tyloses being formed as a vital reaction of the host to injury, mechanical or fungal, since the artificial production of "gumming" by the injection of toxins (3) and the considerable increase in the specific gravity of the infiltrated wood are unanswerable arguments against the decomposition origin of "wound gum." White summed up the position by stating that the brown band with its "wound gum" and tyloses may be regarded as the best criterion we have at present as to the parasitic nature of an invading fungus.

Portions of the infiltrated wood of section 2 have been cut out, soaked in water, sterilised and inoculated with *X. polymorpha* mycelium. Growth on this substratum proved to be very scanty, and after 12 months less than 1 mm. depth of the "wound gum" had been digested, which shows that the "wound gum" tends to limit the spread of the fungus in the wood. The *Fomes applanatus* mycelium entering as a heart rot would gradually work towards the periphery, its advancing hyphae stimulating the production of tyloses and "wound gum" from the living wood, while the hyphae behind gradually assimilate and destroy these products of invasion. There is reason to believe that the *Fomes* rot proceeds slowly over a number of years, being delayed probably by the host's reaction. As all the tissue behind the brown zone consists of dead cells, the *Xylaria* on entering would ramify quickly through this tissue and eventually the black plates would be formed. It seems reasonable that the ramifying *Xylaria* mycelium would tend to be checked on reaching the brown zone, and in consequence of the aggregation of hyphae the black line would arise in the position which it is seen to occupy close to the brown zone. It is probable that the *Fomes* mycelium died out before the entry of the *Xylaria*, but in any case no further advance was made by the *Fomes* after the formation of the *Xylaria* black line. Thus it will be readily appreciated how difficult the identification of a rot from zone lines becomes when, as in this case, the zone lines of two different fungi, a parasite and a saprophyte, are found superimposed on each other.

VIII. FORMATION OF THE BLACK LINE.

Infection of a wood block by conidia or ascospores of *Xylaria polymorpha* results in a thin hyaline mycelium which ramifies through the wood. Eventually the hyaline mycelium tends to aggregate, thereby marking out the zone where the black line will appear. From the examination of the black lines in inoculated wood blocks the development of the line can be followed from the early stages, when the brown mycelium occurs in more or less scattered patches, through the period of gradual extension of the bladder hyphae until the complete circumference of an ovoid-shaped body is formed. This ovoid body consists of the black peripheral zone of bladder hyphae and inside a matrix of practically unaltered wood tissues with remarkably few hyphae present. It is important in view of the various functions which have been attributed to black lines, to emphasise that the bladder hyphae do not move from the position in which they are formed. This fact has been established by the observation and measurement of the black circles formed in cotton-wool at the bottom of the culture tubes. In Plate XII, fig. 6, black circles can be seen in the cotton-wool which is practically pure cellulose. These circles represent sections through ovoid bodies, which have been produced by the black plates forming a shell around a matrix of cotton-wool, so that a complete circumference of bladder hyphae has been formed except where further development has been prevented by contact with the glass. In the wood a large number of these bodies is formed, and as their development is limited by the available space they become closely packed and irregular in shape, producing the characteristic double lines where the circumferences of two adjacent bodies approach or a single line where they become contiguous over a part of their area. When a portion of one of these bodies becomes exposed at the surface it gives rise to an effused black mycelium from which develops the stroma or fructification of the fungus and upon which are borne the conidia and the perithecia.

IX. SIGNIFICANCE OF THE BLACK LINE.

The families Diatrypaceae, Diaporthaceae, and Xylariaceae of the Sphaeriales are distinguished by the immersion of their perithecia in a stroma. These three families have been separated, as the Diatrypaceae and the Diaporthaceae are simple stromatic forms with the stromata only slightly raised from the substratum while the Xylariaceae has developed fully individualised fructifications independent of the substratum. It is hoped by a consideration of the black lines produced by the Xylariaceae to suggest that the stromatic relationship of these three families is of a closer degree than was formerly thought to be the case.

The simplest form of stroma is seen in *Eutypa* of the Diatrypaceae where the perithecia are scattered singly but united by a black crust on the surface of the substratum. Ruhland (22) has given the name protostroma to this primitive type of stroma. The advance from the protostroma type is by the invasion of the hyphae and the development of the stroma in the substratum which result in the formation of an entostroma bounded by a black zone composed of swollen hyphae. According to Ruhland (22) *Diatrype disciformis* is one of the simplest of the type which is characterised by the differentiation of the stroma into an ectostroma and entostroma. In this fungus the ectostroma is formed as a plectenchymous disc between the bark parenchyma and the periderm where it raises the periderm into the form of a flat pustule. Eventually with the enlargement of the ectostroma the periderm is ruptured and the ectostroma protrudes and forms conidia around the base. Meanwhile hyphae from the plectenchymous disc which formed the ectostroma have penetrated the bark parenchyma to give rise to the entostroma, which is composed of fungal hyphae and bark parenchyma and is bounded by a black zone. Perithecia are developed in the entostroma, the upper layer of which forms a black sclerotic plate surrounding the necks of the perithecia. The formation of the hard sclerotic plate completes the separation of the entostroma and the ectostroma and shortly afterwards the latter is cast off leaving the entostromatic plate exposed. Ruhland in his study of the stroma-forming Sphaeriales has chosen this formation of ectostroma and entostroma as seen in *Diatrype* as one of the simplest conditions of the diplostromatic group. He suggests that from this stage the general tendency has been the retention of the ectostroma, its reduction and eventually its fusion with the entostroma.

The simpler genera of the Xylariaceae, as, for example, *Nummularia*, show a very close relationship to the Diatrypaceae and Diaporthaceae in the production of a definite entostroma bounded by a black line in the substratum. The entostroma is mainly composed of the parenchymatous tissue with few hyphae, while the upper effused portion that forms the black fructification consists only of fungal hyphae. The perithecia are imbedded in the bark cells and their necks extend through the plectenchyma to the surface. The author has found black lines in the substratum of *Nummularia discreta*, *Ustulina vulgaris*, *Hypoxylon coccineum*, *Daldinia concentrica*, *Xylaria polymorpha*, *X. carpophila*, and *X. filiformis*. These black lines on microscopic examination were found to be composed of tightly packed bladder hyphae, which vary in size in the different genera, and dark-staining pigment filling up the pits and the lumina of adjoining cells. The morphological structure of the black lines in the Xylariaceae, and also, for example, in *Diaporthe*, is so remarkably similar that it is difficult to deny their homology. And since *Nummularia* is regarded as having an entostroma bounded by a black line, it is reasonable to infer that the black lines in the other genera also represent the marginal zones of entostromata in the substrata. A sequence in the development of the "stroma" or fructification can be followed from the *Nummularia* type, where there is a considerable entostroma in relation to the bulk of superficial "stroma," through *Ustulina*, *Hypoxylon* and *Daldinia*, in which the "stroma" assumes a definite form, to the erect, shrub-like fructification of *Xylaria*. It might be expected that there would be a reduction in the entostroma in proportion with the increase in the "stroma." This may be the case in some genera, as for example *Hypoxylon*, but certainly in *Xylaria* the amount of wood bounded by the black lines is immensely greater in volume than the fructifications.

An examination of the genus *Xylaria* throws more light upon the actual morphology of the entostroma. The Tulasnes (27) have depicted a number of species of *Xylaria* with black lines in the substratum, and although they describe *Xylaria* as perennating by concealed mycelium in the wood and mention that the substratum is "marbled by the innate mycelium," they apparently did not correlate the black lines in the case of *Xylaria* with the black marginal entostromatic zone in *Nummularia* and *Diaporthe*. In the case of *X. oxyacanthae* growing on the fallen fruits of *Crataegus Oxyacantha*, mention is made of the fruits being "marbled with black veins within their substance," but no suggestion is made as to their significance.

The Tulasnes described and figured *X. pedunculata* growing on rabbit dung as having a stroma, subterranean, sclerotoid, ovate, globose or very irregular, giving rise to a slender strand which swells up to form a head when it emerges above the dung. The perithecia are usually borne in this head, but the strand or the submerged stroma should they become exposed may bear perithecia as well. It seems, therefore, that there is no inherent difference in the potentialities of the stroma, strand or normally fertile head, but that their position determines their fertility. A terrestrial species, *X. bulbosa*, is also described as having a "tuber of the size of a small nut." It is logical to classify these "stromata," since they are completely immersed in the substratum, as entostromata, and to deduce, since they can bear perithecia, that they have the same constitution as

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EXPLANATION OF PLATES XI—XIII.

PLATE XI.

Fig. 1. Part of a transverse-oblique section of a beech trunk. The irregular black lines of *X. polymorpha* show up clearly. × $\frac{1}{3}$.

Fig. 2. Transverse section of the same beech trunk seven feet from the base. Black lines of *X. polymorpha* can be seen in the heart rot delimiting areas of widely different appearance. Double lines and ovals are particularly numerous. See description in text, pp. 135–6. × $\frac{1}{3}$.

PLATE XII.

X. polymorpha (Pers.) Grev.

Fig. 1. Fructifications in nature on stump of *Acer pseudoplatanus* at the Royal Botanic Garden, Edinburgh. × $\frac{1}{4}$.

Fig. 2. 14-day culture on oat agar with young stroma arising at the centre. Note the lobed margin and radial ridges. × $\frac{3}{4}$.

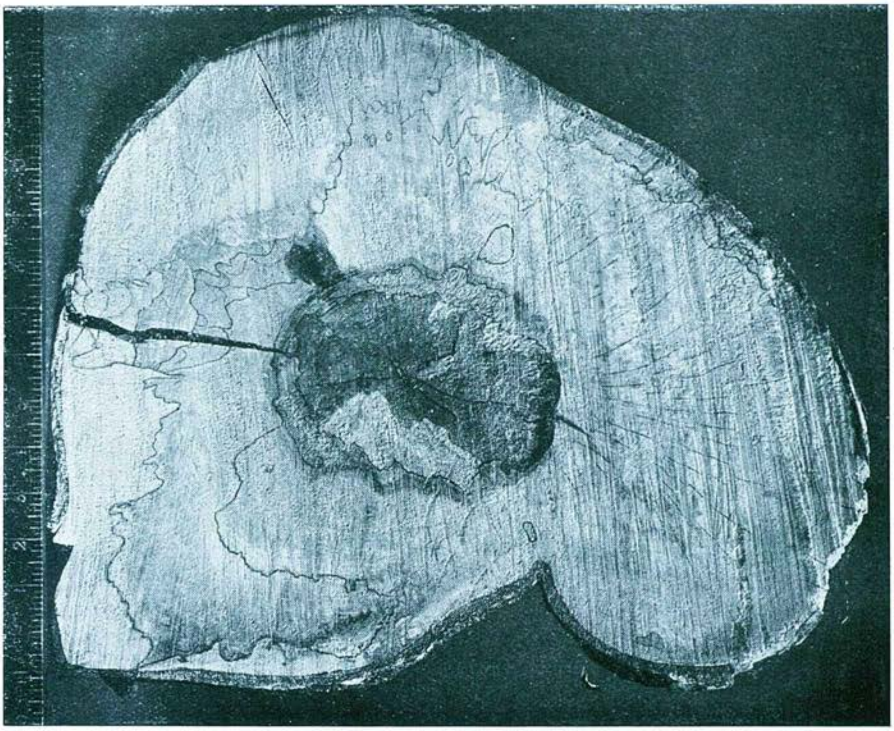


Fig. 2.

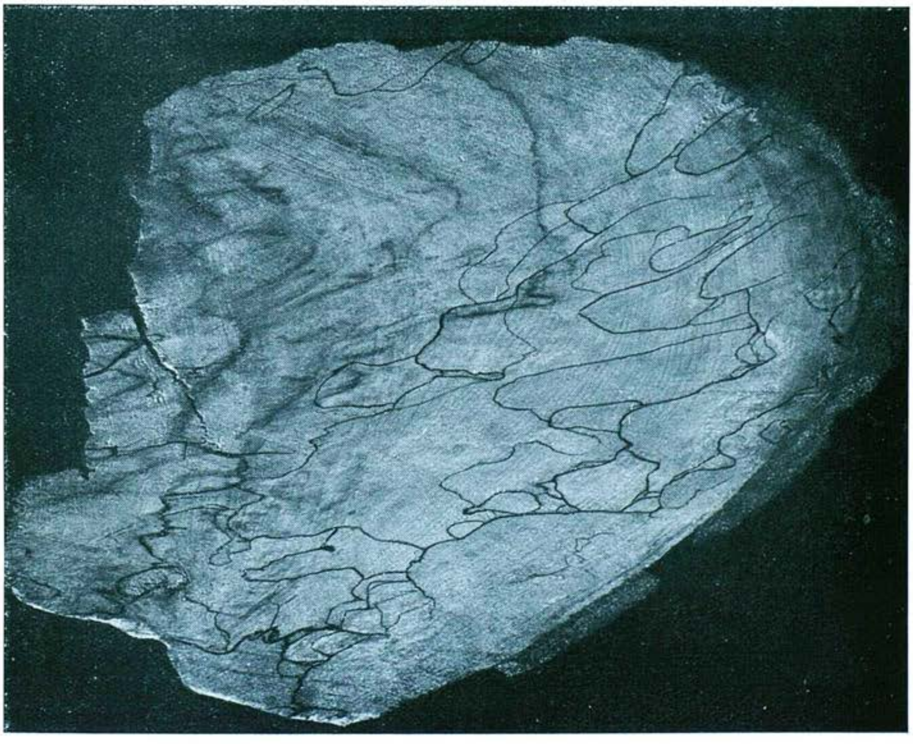
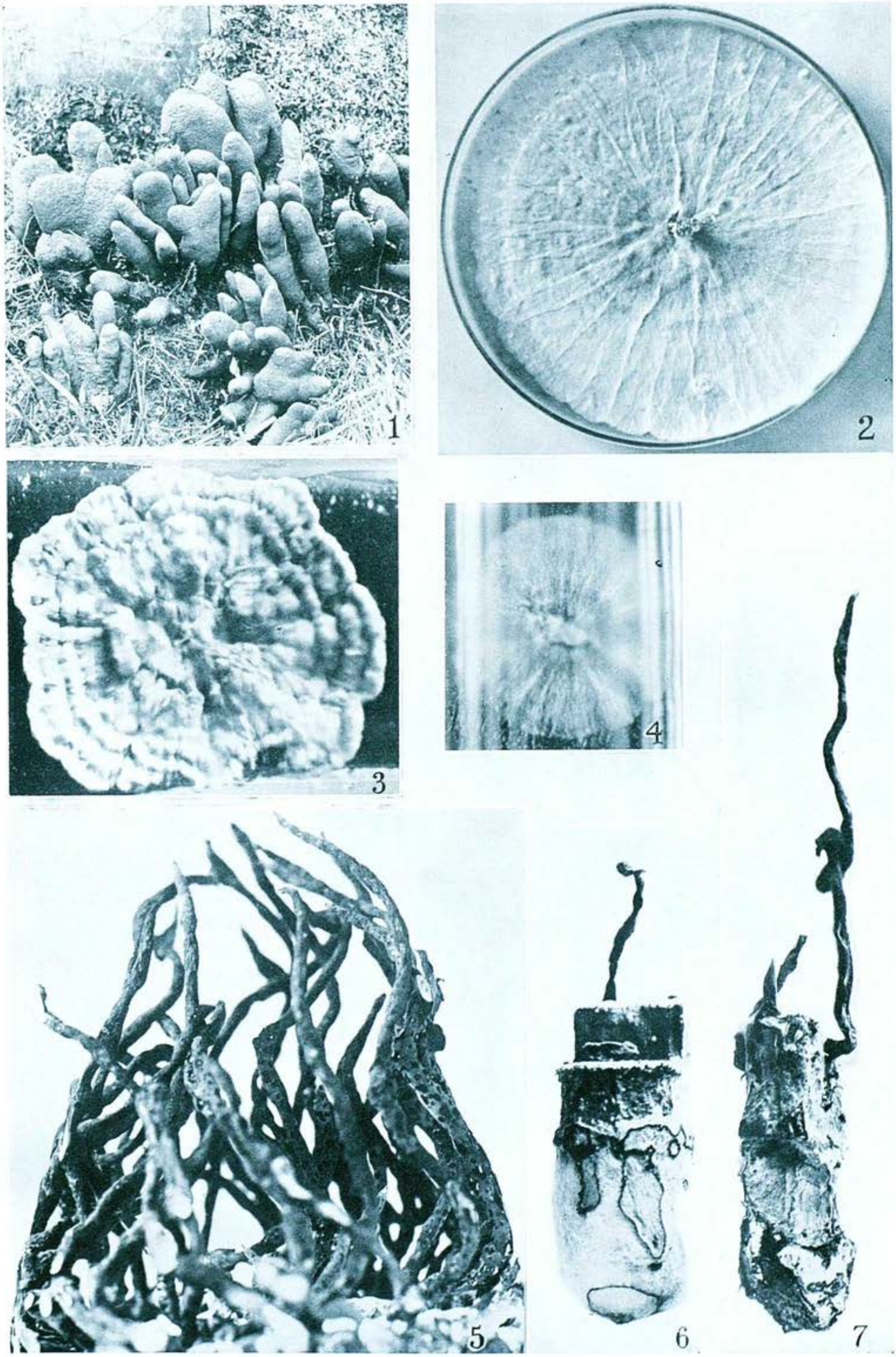
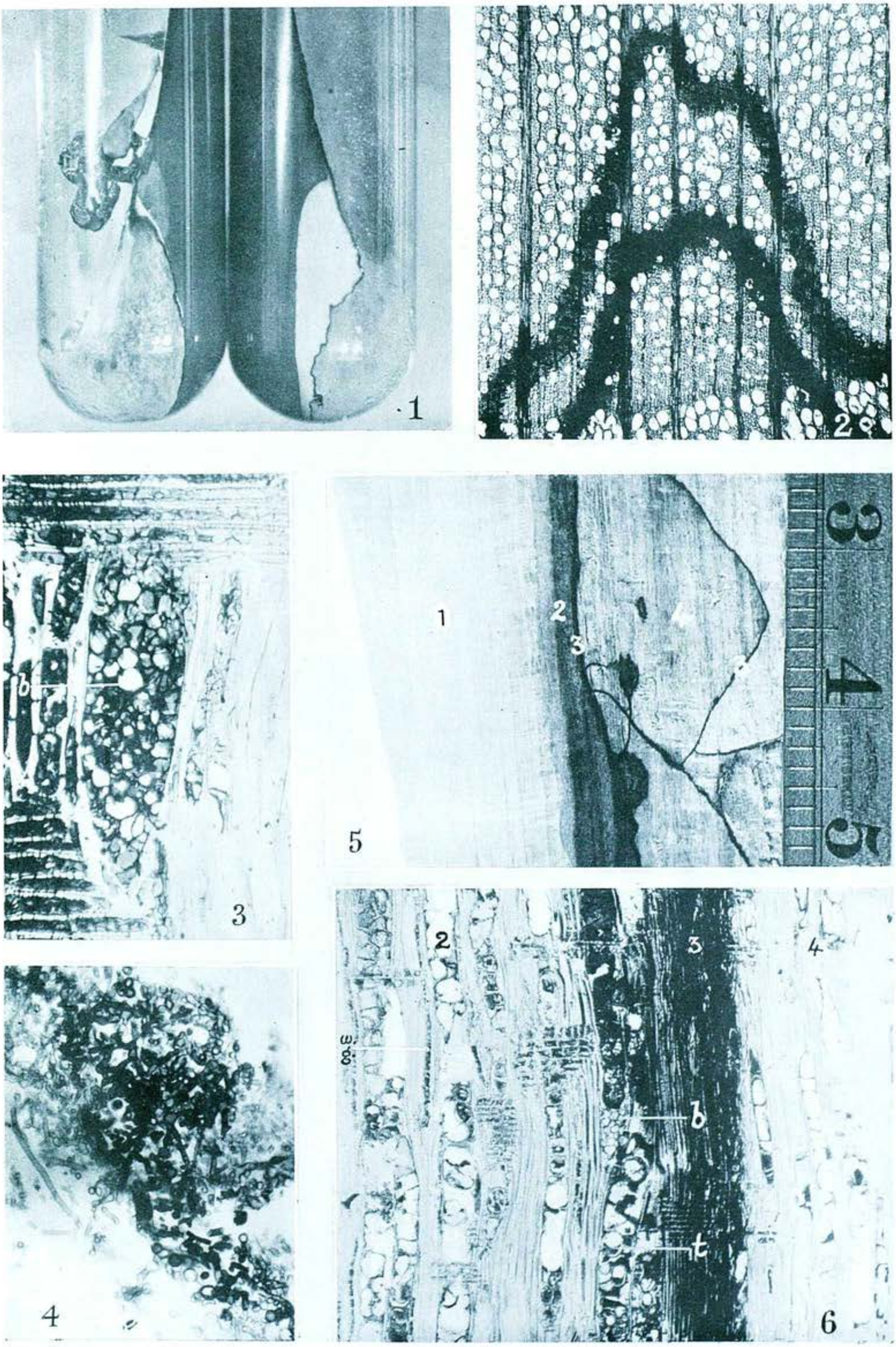


Fig. 1.

CAMPBELL.—ZONE LINES IN PLANT TISSUES (pp. 123–145).



CAMPBELL.—Zone Lines in Plant Tissues (pp. 123–145).



CAMPBELL.—Zone Lines in Plant Tissues (pp. 123–145).

Fig. 3. 7-day culture on sterile carrot block incubated at 25° C. in daylight. The zonation corresponds to the alternate day and night periods. × 2.

Fig. 4. 10-day culture on sterilised beech wood. × 1.

Fig. 5. Group of conidial bearing stromata developed on *C.* medium. The dark patches on the stromata are exuded globules of water. × 1.

Fig. 6. Culture on sterilised lime wood showing a stroma and the black ovoid bodies developed in the cotton-wool at the foot of the tube. × 1.

Fig. 7. Conidial bearing stromata developed on sterilised elm wood. The growth of the long stroma was checked by dropping the temperature from 25° to 18° C. and in consequence a new growth has arisen just below the checked tip causing the malformation seen in the middle of the stroma. Note the production of hairs on the lower half of the stroma by the fall in temperature. × 1.

PLATE XIII.

Fig. 1. Two young cultures of *X. polymorpha* on malt agar. On the left can be seen a young stroma pressed against the glass and on the right a "black line," which is a sectional view of the black incrustation on the medium. × $\frac{3}{2}$.

Fig. 2. Transverse section of beech wood showing double black lines. The irregular course of the black line with reference to the wood tissues can be followed. × 25.

Fig. 3. Longitudinal section of black line in beech wood. The vessels can be seen filled with bladder hyphae (*b*), while the thinner hyphae are found in the tracheides and cells of the medullary ray. Note the staining of the pits of the medullary ray by the brown pigment. Unstained. × 400.

Fig. 4. Section of the black incrustation formed by *X. polymorpha* in culture showing the early stages in the formation of the dark bladder hyphae. Some of the thinner brown hyphae with well-marked septa can be seen. Unstained. × 300.

Fig. 5. Beech wood attacked by *F. applanatus* shown in longitudinal section. The normal uninfected wood (1) is separated from the typical rot (4) by a band of "wound gum" (2), which is produced as a result of the *F. applanatus* attack. In addition, black lines and ovals of *X. polymorpha* (3) are superimposed upon the *Fomes* rot. × 1. Scale in inches.

Fig 6. Longitudinal radial section through the above specimen. No sound wood is shown in this section but the figures 2, 3 and 4 refer to the same areas. The bladder hyphae (*b*) can be seen filling the remaining spaces in the vessels left by the tyloses (*t*), which have become filled with "wound gum." The fractured deposits of "wound gum" (*w.g.*) can be seen filling the lumina of the tracheides. Unstained. × 60.

(*Received May 6th*, 1932.)

ZONE LINES IN PLANT TISSUES.

II. The black lines formed by

Armillaria mellea (Vahl) Quel.

by

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(from the Mycology Department, University of Edinburgh)

CONTENTS. page.

I. Armillaria mellea (Vahl) Quel.

Armillaria mellea (Vahl) Quel., one of the common- /est and most destructive of parasites, is unique in combining an extensive geographical distribution, tropical and temperate, with a degree of polyphagy unequalled in the parasitic fungi. This fungus has attracted so much attention from both the systematist and the pathologist that, since the description of Agaricus melleus by Vahl (15) in 1777, there has arisen a wealth of literature on the morphology, physiology and pathology of the parasite. Within recent years the literature has been augmented by pathological accounts fom North America, Africa, Australia, India and Japan. In addition, the fact that this fungus is one of our commonest tree parasites makes it too well known to need any further introduction. Innumerable figures have been published of the medium-sized toadstool which comprises its sporophore while its characteristic rhizomorphs are even more familiar. The rhizomorphs consist of long black strands, rounded or sometimes flattened like bootlaces, which spread throughout the soil and bring about the distribution of the parasite. They are found commonly below the bark of trees killed by the fungus and later radiating in the soil from the dead trees which thus provide bases/

bases of attack from which the spread to the neighbouring plants is directed.

The spread of Armillaria mellea is remarkable for the variety of plants attacked, for while numer-/ous coniferous and dicotyledonous trees both tropical and temperate succumb to the fungus, it has also been recorded parasitising such varying hosts as heather (1), potato (17), iris (16) and strawberry (18). Although the fungus is so polyphagous not all of the apparently susceptible plants with which its rhizo-/morphs come into contact are infected. To explain this phenomenon the possibility that there are distinct physiological strains has been mooted. Such a case has been described by Childs and Zeller (6) who have shown that in California orchard trees, which are usually very susceptible to Armillaria mellea on oak-cleared ground, are not infected by the fungus on fir-grubbed land although the crowns of the trees were emmeshed in rhizomorphs, which were giving rise to clusters of sporophores. These trees were examined over a period of years and attempts were made to cause infection by wounding and sawing-off roots but none resulted although the wounds were grown over by the rhizomorphs of the fungus. In consequence the authors have suggested that there are two physiological strains, one of which naturally infects the oak while the other is/

is found on conifers, chiefly Douglas fir. From the point of view of parasitism of orchard trees, the oak strain is said to be virulent while the fir strain is not. There is, however, no morphological difference discernible in the two strains which are therefore called physiological strains. This part-/icular problem is at present under investigation but it does seem that more evidence will be necess-/ary to establish the existence of these strains as parasitic virulence to orchard trees has already been attributed to Armillaria mellea originating from coniferous hosts (12). It may be, however, that an examination on the broadest possible lines of the problem of immunity to this fungus will yield a satisfactory explanation of this curious phenomenon.

The zone lines of Armillaria mellea have been found in the wood of the following hosts:- Abies noblis, Fagus sylvatica, Fraxinus excelsior, Larix europea, Picea excelsa, Pinus sylvestris, Pyrus aucuparia, Pseudotsuga Douglasii, Tilia europea, Ulmus montana and Veronica propinqua. These lines, which vary in colour from light brown in the early stages to black in the older rots, were associated in every case with rhizomorphs and in some instances fructifications were present. A number of isolations were made by dissecting out in a sterile manner pieces of the black line and transferring them to malt/

malt agar. As the resultant growths were found to agree within the limits of cultural variation, only a single strain isolated from _Veronica propin-/qua_ will be dealt with in detail and may be regard-/ed as typical of the isolations.

The infected _Veronica_ from which the isolations were made was a small shrub some two feet in height which was dying off in a characteristic manner from the centre as a result of all the main roots being attacked (Plate I, fig.1). The plant, however, was still alive at the margins where, by means of advent-/itious roots, it had been able to establish a food supply. Rhizomorphs of _Armillaria mellea_ were plentiful in the soil around the bush and could be actually traced entering the roots where the black lines were numerous in the bark and wood (Plate I, fig.5). It is believed that the original host of the _Armillaria mellea_ was oak.

In the attacked roots the black lines were continuous in both wood and bark as there was no apparent xylostroma occurring in the position of the cambium. Areas of white mycelium which might be regarded as the xylostroma were to be found, however, in the outer layers of the bark. A transverse section across the root revealed a number of black circles some of which included both wood and bark tissues. Successive sections showed that the/

the black circles were sections across elongated oval bodies which had the black line for their circum-/ference. A number of these bodies of various sizes and shapes but always with their long axes parallel to the vertical axis of the root were found imbedded in the tissues. The wood itself was very white in colour and had obviously been considerably delignified. In the advanced stages of the decay the tissues were so soft and wet that water could be literally wrung out of such infected roots. The black lines, however, remained intact and were rendered even more distinct by the rotting of the surrounding wood which caused the lines to stand out in relief.

II Development of the fungus in culture.

When a piece of wood infected with Armillaria mellea is inoculated on to an artificial medium such as oat agar the hyphae grow out from the wood and enter the medium. Within a short time a tuft of mycelium is formed over the wood inoculum. This mycelial mass is at first white but later becomes brown in colour. When the tuft is white the mycelium is composed of hyaline, densely protoplasmic hyphae which vary in diameter from 2 - 3.5 μ, have few septa and are seldom branched. After a time the hyaline hyphae become darker in colour with more numerous septa, having now a diameter of about 3 μ. Branches then/

then begin to be formed close to the well-marked septa and give rise to a chain of bladder cells dark brown in colour, which vary in diameter from 6 -14 µ. These bladder cells are thick walled ~~and dark brown in colour~~ and their protoplasmic contents have been replaced by a clear fluid. The intertwining of these chains of bladder cells transform the mycelial mass into the closely knit plate of brown pseudoparenchyma which is so characteristic of the fungus when grown on oat agar. This brown plate or tuft, which Brefeld (3) regarded in his cultures as a sclerotium, then gives rise to rhizomorphs. These are usually develop-/ed from the under surface of the plate, that is the side next to the medium, and frequently all of them arise from a single point. The rhizomorphs thus form-/ed grow down into the medium for some distance and then emerge above the surface of the agar. While submerged in the agar the rhizomorphs are flattened and white in colour and are surrounded by a weft of hyaline hyphae which grow out from the rhizomorph into the medium but immediately the rhizomorph emerges above the surface of the medium it becomes cylindrical and dark brown in colour as the surrounding hyphae disappear and in their place is formed the glossy, dark brown rind of the typical rhizomorph. The hyaline hyphae, however, spread along the surface of/

of the medium from the point where the rhizomorph has emerged and form a new mycelial plate which eventually joins up with the original tuft (Plate II, fig.5). Thus in old cultures on oat agar the entire surface is covered with a brown mycelial plate which shows very marked zonation.

The growth of Armillaria mellea in culture varies considerably on different media but, in general, media containing easily obtainable carbohydrates result in the best growth. Such a medium as C medium (5) or bread soaked in malt extract solution are very suitable for producing a large amount of vegetative growth and abundant rhizomorphs which may attain a breadth of over 1 inch. When this fungus is grown on malt agar the mycelial growth is much scantier and is confined almost entirely to within the medium where it forms a black mass below the surface. The rhizomorphs, too, are black in colour and much more natural in appearance. A curious feature of the cultures is that bodies resembling sclerotia may be formed in the depths of the medium as is seen in Plate II, fig.6. It can be seen that this body is very similar in structure to a sclerotium having a white medulla and being surrounded by a black rind except where in contact with the glass. From these bodies in the depths of the medium arise the rhizo-/morphs which penetrate to the surface and grow out free/

free from the medium. Another curious phenomenon in connection with these sclerotium-like bodies is seen in older cultures. Plate II, fig.2 shows the reverse of two slope cultures of Armillaria mellea which have been drying out and in consequence there has been formed a black plate down through the medium to make contact with the glass, thus enclosing the whole of the bottom portion of the slope within a black rind except, of course, where the slope was in contact with the glass. It can be seen that this black plate, which is composed of bladder hyphae, has greatly diminished the rate of drying of the enclosed portion of the medium and thus seems to suggest the formation of a sclerotium which contains, in part, the matrix upon which it is growing.

The mycelium in the cultures on malt agar is gener-/ally formed within the medium but when exposed at the surface it has a black shiny appearance quite different from the cultures on oat agar. The brown hyphae formed in the medium, too, are different from those formed on oat agar as they are generally stouter, more irregular in shape and frequently branched.

Bladder cells are also formed but they are much smaller than normal, being only on an average about 6 μ in diameter, and filled with densely granular protoplasm. There can be no doubt that these hyphae are abnormal forms/

forms as a result of their submergence in the depths of the medium. White mycelium is rare in malt agar cultures and is only seen in the early stages and as a medulla in the sclerotium-like bodies in the medium.

An interesting phenomenon is seen when the tips of rhizomorphs developed in culture are cut off and transferred to a fresh medium. The rhizomorph tip about 1/8th in. long is laid flat on the medium and immediately, without any apparent connection with the substratum, the tip continues its growth so that at the end of ten days the rhizomorph has become about an inch long and has given rise to a number of branches About this time the connection with the medium becomes evident to the naked eye as a dark halo around the point of inoculation but it is not very marked and in no way resembles the tuft or mycelial plate in which the rhizomorphs usually have their origin. Thereafter while the apical growth continues vigorously more and more branches appear until there is a network of rhizo-/morphs spreading over the medium. No aerial mycelium other than rhizomorphs can be seen in such a culture on malt agar but the rhizomorphs may penetrate into the medium for part of their course. This is seen in Plate II, fig.1 where the rhizomorphs in the medium are seen as dark blurred areas due to dark hyphae growing/

growing out from the rhizomorph. The white spots are young branch initials, consisting of a tuft of floccose hyphae as is produced in nature, which were being produced abundantly when the culture was photographed. This elongation of growing tips is an extraordinary feature and can be carried out at will. The pieces for inoculation are cut from the tips of aerial rhizomorphs formed in culture and laid on the surface of the culture medium. In the course of a day or two the rhizomorph will be seen to have perceptibly lengthened and still have re-/tained its distinctive characters as a rhizomorph.

III. Black lines produced in inoculated wood.

Armillaria mellea has been grown in pure culture on various wood substrata in a convenient form of culture such as that shown in Plate II, fig.4. This type of culture consists of a slip of wood placed in a test-tube into the bottom of which is poured a quantity of malt agar. The agar, when it cools, forms a convenient surface for the inoculation of the organism, while its close contact with the wood ensures that the fungus will quickly establish itself in the wood tissues. This form of wood culture has been very sucessful in raising pure cultures on wood from single spore inoculations. The culture shown/

shown in this photograph was derived from the inoculation of a piece of _Armillaria mellea_ mycelium on to the malt agar, where the fungus formed a sclerotium-like body at the surface of the medium. Numerous white rhizomorphs can be seen to have arisen from the lower surface of the body and to be spreading throughout the medium. A single rhizomorph, cyl-/indrical and black in colour, can be seen to have arisen from the top of the body and to be growing up the surface of the wood. The rhizomorph is very similar to those found in nature and is giving rise to a number of branches which appear as white tufts, while the discoloration of the wood shows that a certain amount of attack by the rhizomorph has taken place.

In the cultures set up to test the production of black lines by _Armillaria_, larger pieces of wood were used and the inoculum was placed directly on the surface of the wood. Some device to prevent the cultures from drying out too soon, such as cotton wool soaked in water or a quantity of water in the lower portion of a bulb tube, were included in these cultures which were incubated for a period of 9 -12 months. The woods used in the cultures were Scots pine and ash and they were inoculated with two sep-/arate isolations of _Armillaria mellea_, one from Veronica/

Veronica and the other from strawberry.*

It was evident from observations on the inoculated wood blocks that the mycelium immediately entered the wood at the point of inoculation instead of spreading over the block and giving rise to a mass of superficial mycelium, such as is commonly the case in wood cultures. Subsequent examination showed that the entry was mainly by the medullary rays where there was a considerable number of fine hyphae in the cells. Thence the spread was in all directions through tracheides, parenchymatous cells and fibres, in the walls of which numerous bore holes could be seen. All the inoculated blocks showed the black lines.

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* The author is indebted to Mr. J. A. Macdonald, Edinburgh and East of Scotland College of Agriculture for the use of this strain of Armillaria mellea. The isolation was made from strawberry plants which had been obviuosly attacked by rhizomorphs resulting in the formation of broad bands of white fungal plectenchyma in the roots. No black lines or rhizomorphs, however, were observed within the soft tissues of the root.

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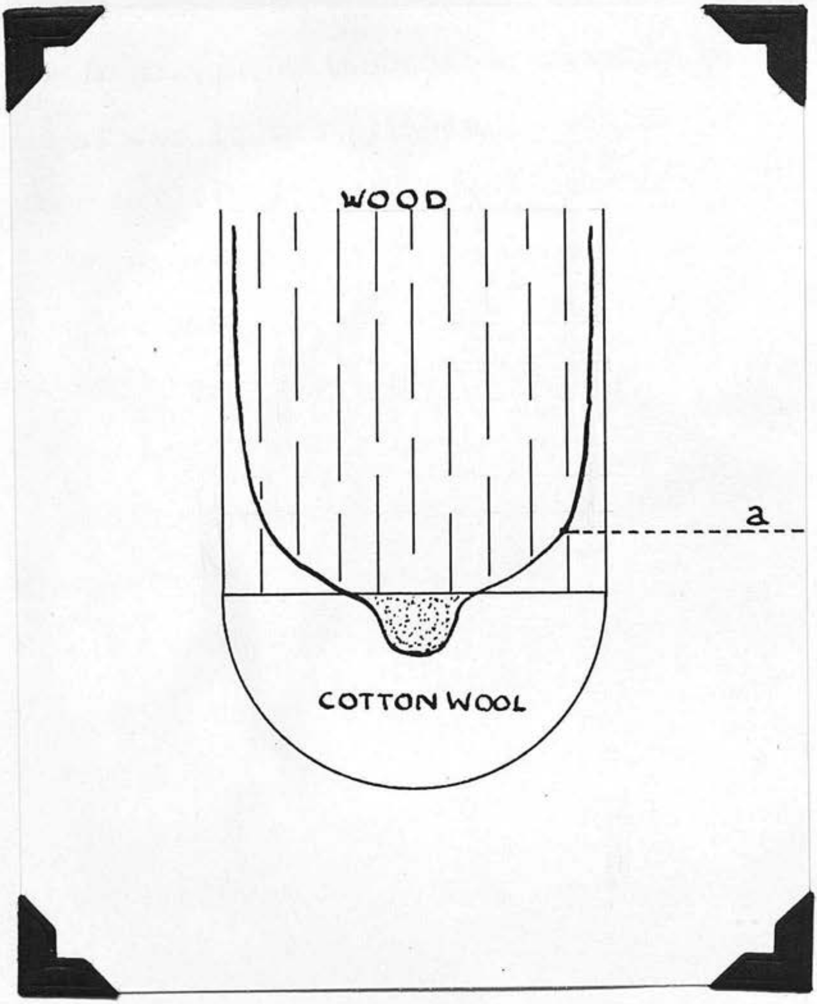

Text figure 1.

Showing the formation of the black line (a) in an inoculated wood block. The black line has been continued into the cotton wool upon which the block was resting in the culture tube and has formed there a structure comparable to the black xylostroma in nature.

Generally, these black lines occurred as the periphery of a large ovoid body occupying the centre of the block (Plate II, fig.3) but in some cases the lines were quite superficial. A common feature was the formation of a line across the "end wood" some 1/16th in. below the cut surface. In one such instance (text fig.1) the brown line formed beneath the surface of the "end wood" had been extend-/ed into the cotton wool upon which the wood was resting and formed there a structure closely resembl-/ing the black xylostroma that is formed below the bark of trees attacked by <u>Armillaria mellea</u>. The interior of this structure was composed almost entirely of cotton wool with a compar~~i~~atively small bulk of fungal mycelium, whereas the black xylostroma in nature has a solid white medulla of fungal plecten-/chyma.

Examination of the culture blocks at varying intervals showed that the method of formation of the <u>Armillaria</u> black lines is very similar to that of the <u>Xylaria polymorpha</u> lines (5). The position, which the line is to occupy is first marked out by aggreg-/ations of thin hyaline hyphae. These hyphae swell up to assume the bladder form and are at first colour-/less but later become dark brown in colour. The bladder hyphae tend to be formed in patches which spread/

spread outwards until they meet with the adjoining patches and thus the complete line is formed. The bladder hyphae are found only in the lumina of the cells and these isolated clumps are connected by a thin hyaline mycelium, which passes from cell to cell by small bore-holes as there is little or no delign-/ification of the cell walls at this time. Indeed, the presence of the bladder hyphae pressed close to the walls so far from bringing about their disintegr-/ation actually preserves them, so that, in the later stages of decay, the few sound cell walls are those to which the bladder hyphae have been closely applied. It is important to remember that these bladder hyphae are not continuous but that they occur in clumps which serve to plug the cells in which they are formed. The only connection such a plug has with those in the tracheides around it are is by means of a few slender hyphae penetrating the walls. The parts, then, of the tracheide walls incorporated in the line form in the aggregate a considerable bulk of the structure and assist materially in the formation of an impenetrable barrier.

The brown pigment of the bladder hyphae is very similar in reaction to the pigment in _Xylaria_, although the _Armillaria_ line in its early stages is not nearly so dark in colour. Again, as in the case of _Xylaria_

a/

a certain amount of pigment escapes from the bladder hyphae, stains the walls of the tracheides and fills the pits between the cells of the medullary rays. The bladder hyphae themselves are rather larger than those of Xylaria polymorpha having an average diameter in the Veronica root of about 20 µ for the fully developed bladder cell. The average size of the bladder cells, however, is not constant and may vary considerably in different hosts as, for example, in Douglas fir they are about 18 µ in diameter compared with Scots pine at about 24 µ. In the case of the bladder cells of the black lines, which were formed in the Scots pine blocks in artificial culture by the Armillaria mellea strain isolated from strawberry, a large increase to 35-40 µ in diameter was recorded (Plate III, fig.2). This, however, may be a response to the abnormal conditions obtaining in artificial culture.

IV. Significance of the black line.

The presence of black lines in wood attacked by Armillaria mellea was early recognised and Hartig (10) in 1878 was the first to describe them. He described the black line as being composed of swollen, bladder-like hyphae, brown in colour, which occupied the lumina of the tracheides just as tyloses are found in the tracheides of many dicotyledonous trees.

These bladder hyphae, he stated, were a stage which was highly favourable to the development of the mycelium. In addition, he conceived a sequence of development from hyaline mycelium to bladder hyphae which, when taking place in successive tracheides, would cause the line to advance. He further postulated that the bladder hyphae thus formed soon died off and on digestion were replaced by a delicate filamentous mycelium and thus the black line (i.e. the bladder hyphae in the tracheides) seldom occupied a breadth of more than 3 or 4 tracheides. As a result of the passage of the line the walls of the elements of the wood were stated to display a cellulose reaction and speedily dissolve from the lumen outwards.

According to Hiley's account (11) the honey fungus comes to occupy the cambium and from this position invades the wood on one side and the bark on the other. The extent of the invasion in the xylem is marked by a black line , one or more trach-/eides thick, and generally in the shape of a tri-/angle with its base in the cambium. He came to the conclusion that the bladder hyphae composing the black line represent a stage of excessive vigour in in the mycelium which, although it does not directly cause/

cause marked changes in the wood, seems in some way to transform the wood into a state in which it is easily acted upon by the hyphae behind. In addition, he further elaborated Hartig's account of the move-/ment of the line and illustrated the mechanism by which this phenomenon was brought about. He differs, however, from Hartig by his statement that no percept-/ible difference can be seen in the wood on either side of the black line and by pointing out that no more bore-holes are seen on one side than the other, that the tracheide walls give a lignin reaction and that the tori of the bordered pits remain intact. But he does state that marked delignification occurs some distance behind the black line and attributes this to the action of the black line.

Butler (4) has pointed out that, while the black line may divide the wood into areas that differ in appearance and in the extent of the decay, there is no evidence that the position of the line moves progressively forward. The whole, he declared, is much more like the development of some defensive reaction on the part of the host or parasite.

With regard to the theory advanced by Hartig and Hiley, it must be remarked that if the bladder hyphae are actually "a more active stage in the metabolism of the fungus" then they seem singularly ill-adapted, physiologically, to their function. It is/

is to be expected that hyphae of high functional activity would present the maximum surface area for assimilation and also be well spaced to allow of sufficient aeration. This does not seem to be the case with the hyphae of the black line which are so tightly packed in the tracheide that they immediate-/ly suggest a plug(Plate III, figs. 1-3). The writer (5) has demonstrated before how later sapro-/phytic fungi and bacteria can bring about the difference in decay on either side of the black line of Xylaria polymorpha and there can be no doubt that this is also taking place in the case of Armillaria mellea. It should also be borne in mind that in relatively sound wood Hiley was unable to find any difference in the attck on the wood on either side of the black line. In the course of a protracted examination of black lines caused by Armillaria mellea in the wood of Abies noblis, Fagus sylvatica, Fraxinus excelsior, Larix europea, Picea excelsa, Pinus sylvest-/ris, Pyrus aucuparia, Pseudotsuga Douglasii, Tilia europea, Ulmus montana and Veronica propinqua the writer has in no case been able to discover any evid-/ence of the sequence of events described by Hartig and Hiley which, they stated, resulted in the progress-/ive movement of the line. Nor has the writer found any evidence of these stages in examining material of Hartig's of the black lines of Armillaria mellea in pine/

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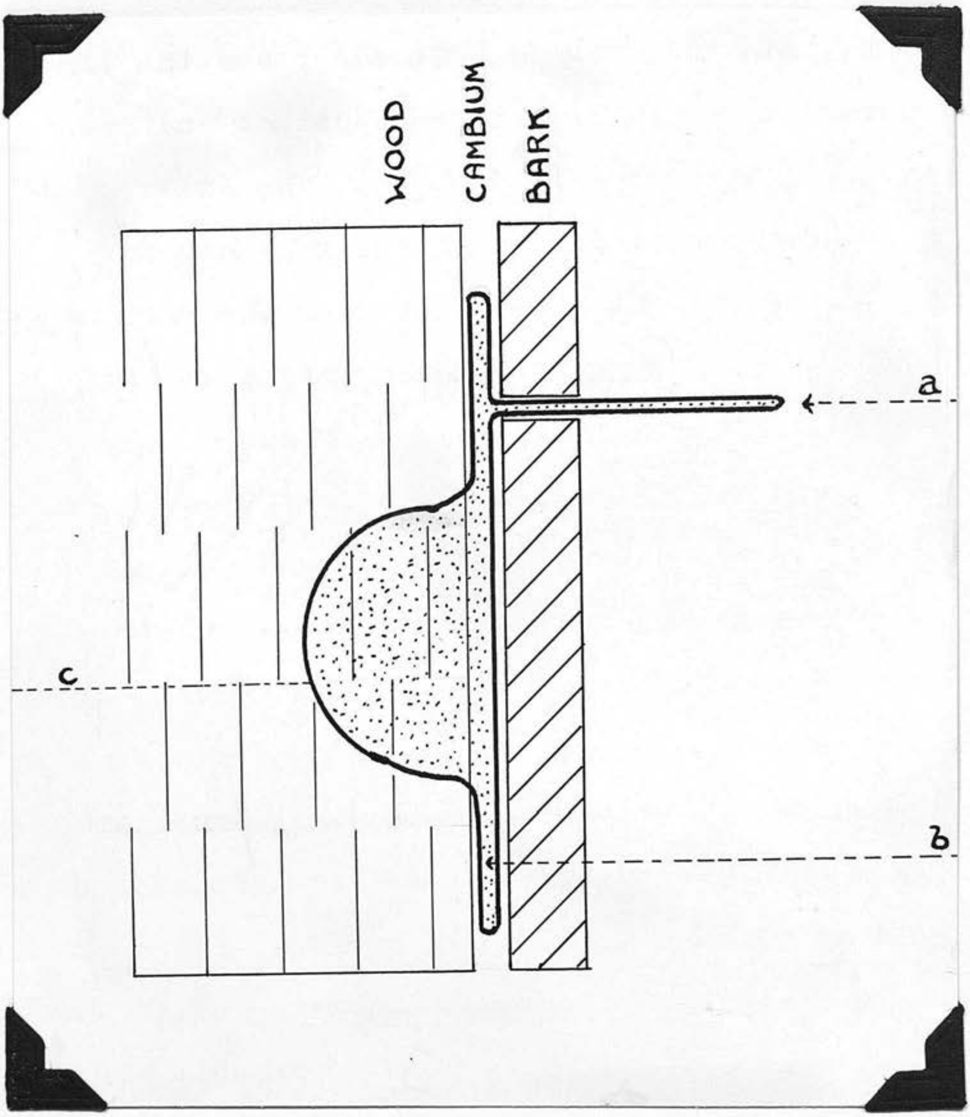

Text figure 2.

A diagrammatic view of the pseudosclerotium as seen in a longitudinal section of the tree. It can be seen to consist of a, Rhizomorpha subterranea; b, the black xylostroma and Rh. subcorticalis; and c, the body bounded by the black line in the wood.

pine in the Museum of the Royal Botanic Garden, Edinburgh (Plate III, fig.1). In all cases the line was observed to be clear cut and distinct and often only a single tracheide in breadth. The walls of the tracheides containing the bladder hyphae are always stained a light brown colour by the pigment from the hyphae so that in this way any movement of the line would be readily detected. Indeed, there is no movement of the line from the position in which it is formed.

The clue to the nature of these lines, however, lies less in their microscopic structure than in their position and in the shapes in which they are found in the substratum. These black lines, of course, are only lines when seen in section and are actually plates or sheets of bladder hyphae. In a transverse section of a root or stem attacked by Armillaria mellea the black plates are generally seen in section as black lines or circles, since the plates mostly occur parallel to the tree axis. Again, as Hiley (11) has pointed out the black lines often occur as a triangle with its base in the cambium. This is, of course, when seen in section but, actually, the black line is in the form of a shallow basin-like structure with the open mouth at the cambium as is seen in Plate I, fig.3 and in text fig. ~~1~~ 2. At the position of the cambium the black plate is continued as a free flattened body, the black/

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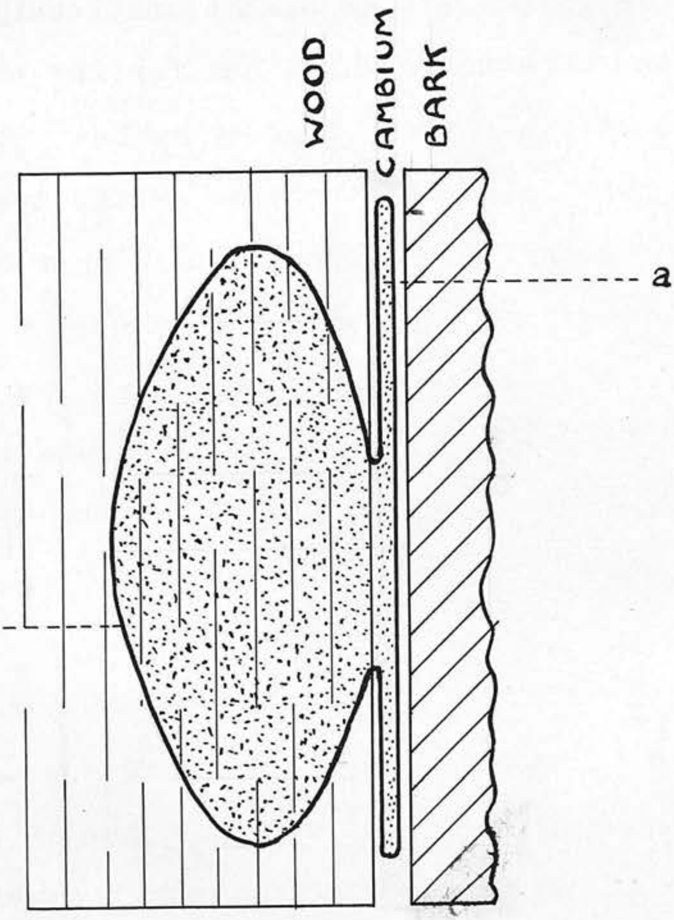

Text figure 3.

Diagrammatic view of the pseudosclerotium found higher up the tree as seen in longitudinal section. It consists of a, *Rhizomorpha subcorticalis* and b, the body bounded by the black line in the wood. Description in text page 20.

black xylostroma, which completes the structure. Thus there is formed a body almost completely submerged in the substratum which has for its periphery the black plate or rind of bladder hyphae. This type of body is common at the base of the tree near the point of entry of the fungus but higher up the trunk the bodies are generally formed deeper in the wood tissues. In this position they are more regular in shape and are seen to be elongated ovoid structures, with their long axis generally parallel to the tree axis, which are buried so deeply in the tissues that only a very small area of their circumference is exposed at the cambium to form the black xylostroma (text fig. 3). Such a body in transverse section appears as a black ring of bladder hyphae in the wood as is seen in Plate III, fig.4. When, however, there is a number of such bodies being formed in the wood their shape is largely determined by the available space, so that generally they become very irregular in size and form. The bodies are all formed quite separately and tend to preserve their individuality to the extent of exerting a repelling influence on each other so that the two opposing black plates come to be formed always at the same distance from each other, which is usually a few mms., and thus produce the characteristic double or parallel lines. In many cases these bodies may be formed in the bark as well as/

as in the wood and, when the attack on the cambium has not been severe enough to cause the breaking away of the bark from the wood, the bodies may be formed partly in the bark and partly in the wood. Indeed, since the black plates are formed through all kinds of tissues, a single body may include within it a representative collection of all the tissues of the host.

It seems, therefore, that the black plate functions as a limiting layer or rind to the bodies in the wood. As has already been pointed out, this rind by nature of its closely packed hyphae presents an impenetrable barrier to the entry of later organisms occuping the same substratum. Another still more important function is that the bounding layer acts as a watertight envelope, which prevents the drying out of the tissues within it, and thus enables the body to withstand conditions of dessication which would kill mycelium not so protected. Further, the structure of the plate of bladder hyphae is so similar to the rind of the rhizomorph and the wall of many sclerotia that it must be regarded as homologous in character and as forming the rind of a sclerotium-like body, which is immersed in the tissues of the host. In the writer's opinion, the fact, that the body is formed almost entirely within the matrix and consists largely of the unaltered tissues of the host, does/

does not prevent it from being regarded as a sclerotium, since, physiologically, it serves the same purpose as the more obvious sclerotium. The peculiar nature of a "sclerotium" buried in a woody substratum, however, has led to further developments for the presentation of the reproductive organs at a suitably exposed position and has brought about some remarkable innovations in the character of the "sclerotium" in the wood. The exact nature of these changes and the necessity for ditinguishing morpholog-/ically this "sclerotium" in the wood from the true sclerotium will be discussed in the next section.

V. Nature of the sclerotium.

"A fungus, it seems, never begins with a sclerotium; at least we know no fungus whose seeds on beginning to germinate grow at once into so many sclerotia". Tulasne (14).

"All sclerotia, it would appear, develope as secondary formations on a primary sporogenous fila-/mentous mycelium". De Bary (2) 34.

The sclerotium, then, is a secondary mycelial condition, following the establishment of the primary mycelium, whereby the reserve food-materials are stored in a tuber-like structure to await their util-/isation in the formation of reproductive organs. Sclerotia are literally storehouses of food in the concentration/

concentration of which there is usually a copious exudation of water from the structure. Typically, the sclerotium consists of a uniform white tissue, the medulla, which is surrounded by several layers of large darker-coloured cells which compose the rind. The rind in most cases becomes black in colour and the mature sclerotium, which has a definite outline and is commonly spherical in shape, generally becomes detached from the mycelium upon which it was borne. Thus the sclerotium is best regarded as a stage in the life-history of the fungus, intermediate between the formation of vegetative mycelium and fructification, when the thallus prepares for the reproductive phase. After remaining dormant for a period the sclerotium germinates by the formation of fructifications of the apothecial, perithecial and basidiomycete types but the sclerotium of _Botrytis cinerea_ produces only simple conidiophores on germination. This is, however, an exceptional case.

The sclerotium may be borne free on the surface of the substratum or, as is more commonly the case, either wholly or partly immersed in the tissues of the host. In this latter case pieces of the matrix in which the sclerotium was formed are frequently found within the medulla. Corda (7) has shown that sclerotia like that of _Sclerotinia Sclerotiorum_ often enclose portions of the tissues of the plants, which they/

they inhabit, in their own substance, while De Bary (2) fig. 19. has figured dead cells of the vine leaf in the sclerotium of S. Fuckeliana. S. Candolleana may also include parenchymatous cells of the oak leaf within its sclerotium. The presence of host tissues within a sclerotium does not make it necessary to distinguish it as a new type of sclerotium as, indeed, a number of the sclerotia of the Agaricaceae contrive to include within their substance a varied collection of stones and organic debris. The criterion of a true sclerotium is, in the writer's opinion, whether or no the sclerotium is a separate entity with no organic connection with the substratum at the time of germin-/ation. Thus the sclerotium, which is borne free on the surface of the substratum or is subsequently set free by the disintegration of the tissues in which it was formed, is a true sclerotium.

VI. The pseudosclerotium.

This investigation on black lines has led to the recognition of a number of sclerotium-like bodies in woody tissues. These are very largely imbedded in the woody substratum where they are only recognis-/able by the black lines or plates which comprise the rinds of the bodies. No medulla of fungal plecten-/chyma is visible to the naked eye but in its place are the almost unchanged wood tissues of the substratum.

Part, however, of this curious sclerotium-like body may be formed on the free surface of the substratum where it has been called the black xylostroma. This black xylostroma immediately suggests on examination its affinity to the true sclerotium; being formed of a white medulla of fungal hyphae and surrounded by a rind of dark-coloured bladder hyphae, which is con-/tinued in the wood as a black line or plate. There can be little doubt that this body within the wood fulfi~~l~~s the same physiological requirements as the true sclerotium and, further, there is evidence that the fructifications arise only from such a body or its extension. It differs, however, from the true sclerotium in the perennial nature and curious plast-/icity of the body which has doubtless arisen from its immersion in woody tissues. It is not advisable, therefore, to include this body in the wood within the term sclerotium on morphological grounds and, in consequence, it is proposed to introduce the term pseudosclerotium, which will distinguish it morphol-/ogically and yet suggest its affinity to the true sclerotium.

The Xylarias provide good examples of these sclerotium-like structures formed in the substratum which, in order to establish their connection with similar structures occurring in members of the Diatrypaceae and Diaporthaceae, the writer had pre-/viously/

previously named entostromata. In many ways the term pseudosclerotium is more comprehensive and capable of a wider application than entostroma to which it must be preferred if only on the grounds that the term stroma has in the past been applied to so many fundamentally different structures. In the case of Xylaria polymorpha the pseudosclerotium is almost entirely buried in the wood substratum but it has the black xylostroma as a free extension beneath the bark. This xylostroma each year develops a number of growing points which grow out from below the bark as the fructifications or "stromata" to bear first the conidia and later the perithecia. These fructifications are simply extensions to and part of the pseudosclerotium in the wood and their structure is very similar to that of the true sclerotium. They have, however, acquired the power of apical growth, which is sufficient to distinguish them from the true sclerotium. This development of apical growth is probably a result of the pseudosclerotium being buried in the wood and the necessity for the reproductive organs being borne at a suitably exposed position on the surface. These fructifications, which arise by apical growth from points on the xylostroma, do not generally grow out more than a few inches but in culture their/

their length and shape can be considerably modified and, in particular, the exudation of water from the fructifications is strikingly reminiscent of the formation of true sclerotia.

The case of Armillaria mellea is strangely parallel to that of Xylaria. Again, there is a pseudosclerotium buried in the wood with a free extension of black xylostroma (including Rhizomorpha subcorticalis) below the bark from which the rhizo-/morphs (Rh. subterranea) arise by apical growth in a manner comparable to the fructifications of Xylaria. The rhizomorphs of Armillaria mellea, however, are possessed of the power of unlimited growth, so that not only do they grow out to a suitably exposed position but they continue to elongate and spread throughout the soil on all sides of their host. But they still remain simply an extension of the pseudosclerotium in the wood and as such still retain the sclerotial function of bearing sporophores. These fructifications may be borne only on the xylostroma, chiefly at the crown of the tree, or, as has been figured by Hartig (10), ~~and~~ Hiley (11) and others, on the rhizomorphs in the soil. As a result of the ramifications of the rhizomorphs in the soil, it is inevitable that they will come into contact with the crowns and roots of other plants whose/

whose outer cork layers they are able to penetrate as a whole and establish themselves in a new host by giving rise to a vegetative mycelium. This formation of vegetative mycelium is a new method of reproduction for the pseudosclerotium and, probably, has been developed as a corollary of the unlimited apical growth of the rhizomorphs. The extension of the pseudosclerotium as a rhizomorph, however, is not altogether a new development and is foreshadowed in the formation of the Xylaria fructification. Indeed, the rhizomorphs when limited in growth, such as Dade(8) has described as frills of black xylostroma of Armillaria mellea on cacao in the Gold Coast, are very similar in appearance to the fructifications of Xylaria polymorpha. But, it is surprising that this new method of vegetative growth of the pseudosclerotium should be so successful as to oust the sporophore from the position of chief organ of reproduction.

VII. Biology of Armillaria mellea.

The biology of Armillaria mellea so far as it concerns the development of the fungus within the host is not well known. The accounts are incomplete and do not deal fully with the important stages lead- /ing/

leading up to rhizomorph formation. In addition, the new light thrown on the biology of the fungus by the description of the pseudosclerotia in the wood necessitates a new and fuller account of this phase in the life-history of the fungus. So many accounts of Armillaria mellea have been published that the writer feels, that, with the exception of the pseudosclerotium, the various manifestations of the fungus within the host have already been suffic-/iently illustrated. To reproduce this material would be superfluous so that the writer will refer as far as possible to the original descriptions and only attempt to supply an interpretation of the various stages which have been so described. The fundamental idea behind this interpretation is the distinction between the vegetative and reproductive phases of the mycelium. The vegetative mycelium is composed of thin hyaline hyphae, which bring about the death of the cambium and the delignification of of the woody tissues. Following this initial phase comes the stage when the mycelium alters and becomes reproductive in character forming the pseudosclerotia. These pseudosclerotia are adapted for reproduction either by the production of sporophores or by the direct penetration of a new host and the formation of/

of the vegetative mycelium within it.

The methods of infection employed by Armill-/aria mellea have never been made the subject of detailed study although various writers, at one time or another, have stated how they believe the infection to be brought about. It is generally agreed that the basidiospores play no part in the infection of living plants and that they are con-/cerned only in the infection of dead stumps in which the mycelium will eventually produce rhizo-/morphs capable of attacking living hosts. The rhizomorphs, which are believed to be the main instrument of infection, have been the subject of considerable controversy as to whether they can penetrate the healthy uninjured bark and gain access to the living tree or whether they can only attack through wounds and dead tissues. As long ago as 1877, Brefeld (3) showed that the rhizomorphs could penetrate the living bark of roots, which had been freshly dug up, and thus bring about their infection. Hartig (9) also stated that the rhizomorphs bored into the healthy bark and brought about infection but Hiley (11) is of the opinion that the rhizomorphs can only enter the host through dead roots and wounds. Recently Thomas (13) has stated that the invasion of the root is accomplished/

accomplished by the direct penetration of a branch of the parent rhizomorph through the sound healthy cork layer of the host. The method is similar in the various hosts with no apparent difference between susceptible and resistant ones. After gaining entrance, the rhizomorph gives rise in susceptible roots to mycelium which causes the general destruction of the host tissues, while in resistant ones, although the fungus gains entrance, it is unable to establish itself and the wound quickly heals over. In Thomas' opinion the structural and morphological differences in the host exert little influence on resistance, which appears to be in the nature of a vital, antagonistic reaction between host and parasite. From observations on young Douglas fir 6 - 8 years old attacked by Armillaria mellea, the writer has confirmed that entry and infect-/ion in this case was through sound healthy bark as it was possible by dissection to expose the rhizomorphs where they had grown through the bark and given rise to the mycelium in the cambium. Another method of infection has been recorded from the Gold Coast, where Dade (8), working on a collar crack of cacao caused by Armillaria mellea, has stated that the rhizomorphs of the fungus have not been found in nature although they are formed readily in culture. In the cases examined/

examined the infection was due to actual contact of the host's roots with the diseased roots of other plants or with rotting wood. He figures the case of an Eriodendron root, which had been severed from its parent tree, upon which Armillaria mellea was growing saprophytically. Thirty-three yards of this root were uncovered and in its course it was found to have infected six cacao trees by contact with their lateral roots. This method of infection opens up a new field of enquiry and in this connection more details of the actual infection would be valuable.

Infection, then, is brought about by the rhizomorph penetrating the cork layers of the root as an entity and giving rise to a vegetative mycelium which attacks the cambium. This hyaline mycelium, composed of slender hyphae with numerous branches, spreads rapidly in the cambium killing the cells and penetrating on either side into the bark and wood. Shortly afterwards a thin white layer of mycelium, papery in texture and marked by ridges, makes its appearance in the place of the killed cambium and begins to spread upwards and downwards in the root. This thin white sheet of mycelium closely appressed to the wood is a very characteristic symptom of the early attack of Armillaria but it is temporary and disappears in the later/

later stages when the rhizomorphs have been formed below the bark. The term xylostroma has been applied to this white mycelial sheet and also to the black layer below the bark, which is the exposed part of the pseudosclerotium in the wood, and it has been assumed that the white xylostroma by time changes into the black xylostroma. Actually, this is not the case as these two mycelial phases are quite distinct. Some parts of the white xylostroma may never change into black xylostroma as is shown in Plate I, fig.2. where the white mycelium is found alongside the black xylostroma, which has been scraped off to expose the black lines in the wood. On the other hand some portions of the white xylostroma, which have been marked off to form a part of each pseudosclerotium, thicken and eventually develop bladder hyphae until they have formed a complete black rind over the surfaces of the pseudosclerotia. This black rind is continued in the wood as the black plate which delimits the pseudosclerotium. It has been observed in several cases that the black xylostroma and the black lines are always formed more readily where wounds and splits in the bark have exposed the wood and where rapid drying has taken place.

In the examination of the black xylostromata, stages are found where the xylostroma is being resolved into/

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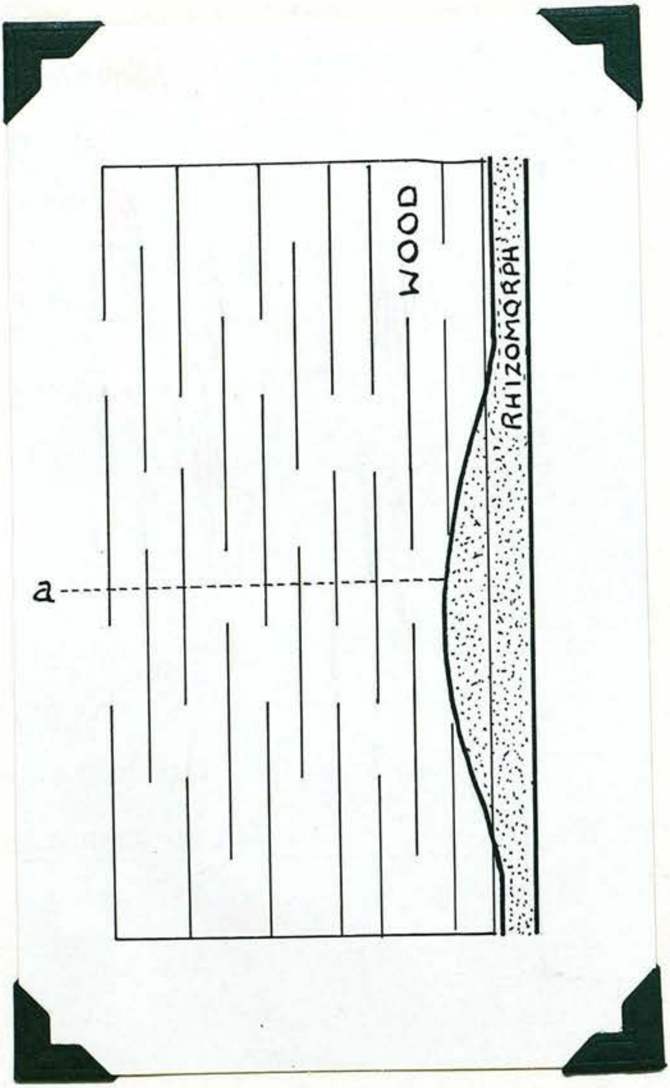

Text figure 4.

Showing the formation of a black line representing the rind of the rhizomorph (Rhizomorpha subcorticalis) which has become partly immersed in the wood.

into the slender flattened strands or the _Rhizomorpha subcorticalis_ of Persoon (Plate I, fig.4). These rhizomorphs are identical with the xylostroma and are simply an extension of that body where the grow-/ing point is confined to the tip of the slender strand. The rhizomorphs by their apical growth spread up the tree following the hyaline mycelium, which has already killed off the cambium and is ramifying in the wood, and occupy the position of the killed cambium between wood and bark. Hyaline hyphae grow out from the rhizomorphs and in this way the rhizomorphs secure a proportion of their food as they ascend the trunk. Sometimes in their passage up the tree the rhizomorphs seem to become partly submerged in the wood so that the lower part of the rhizomorph is represented by a black plate of bladder hyphae in the wood (text fig.4). Meanwhile, the mycelium further up the trunk is constant-/ly forming pseudosclerotia, which differ from the lower ones in having a more definite spindle-shape structure and being buried more deeply in the wood. The periphery of such a body appears at the surface of the wood as a long narrow, black xylostroma, which gives rise directly to a rhizomorph. The pseudo-/sclerotia towards the base of the trunk are shallower or roughly triangular in section, presenting a broad face/

face at the surface of the wood which forms the black xylostromatic mass. In addition to this direct origin of rhizomorphs from pseudosclerotia in the wood, the number of rhizomorphs is considerably augmented by the branching which goes on abundantly if the food supply is sufficient. Altogether this results in a closely woven tissue of rhizomorphs below the bark. Examination of such a weft will not show black lines below all the rhizomorphs. Some, however, will show a definite connection with a pseudosclerotium, if they are carefully examined, but the branching which has been going on has resulted in a number of rhizomorphs which have no obvious connection with the substratum. But it must be remembered that all the rhizomorphs can be traced back to a pseudosclerotium in the wood, however remote it may be.

Eventually, from the flattened rhizomorphs below the bark, branches grow out between the cracks in the bark to enter the soil, where they do not generally penetrate deeper than a few inches below the surface. These rhizomorphs, the Rh. subterranea of Persoon, are now cylindrical with fewer branches and are some-/what smaller than the flattened rhizomorphs below the bark. The flattened shape of the latter rhizomorphs (Rh. subcorticalis) has been previously attributed to the narrow space/

space in which they had to develop and to the pressure exerted upon them by the bark. This may be the explanation but it is interesting to note that in the writer's cultures the rhizomorphs formed within the medium were flattened and band-like. In a very nutrient medium these rhizomorphs attain a size of about 1 in. wide and less than 1/8th in. thick. On reaching the surface of the medium these bands assume a cylindrical shape so that the rhizomorph which projects into the air is always completely cylindrical with a diameter of about 1/8th in. Observations on these cultures seem to suggest that the flattened shape of the rhizomorph may be due to the expansion of the surface area to allow of increased food intake when in the medium, while outside the presence of air and consequential loss of water by evaporation along with the absence of food to be absorbed will result in the reduction of the surface area to a minimum, which will be met by the formation of a cylinder. On making its way out into the soil through cracks in the bark, the rhizomorph prepares to exercise its reproductive function by the formation of sporophores or by the direct infection of a new host. The remarkable feature, however, of the biology of <u>Armillaria mellea</u> is the development of apical growth by which the pseudosclerotium is/

is enabled to bring itself into direct contact with a new host and to implant there its vegetative mycelium. The certainty of this method of reproduct-/ion over any development of spore production and distribution is amply emphasised by its success in establishing Armillaria mellea as, perhaps, our most serious tree parasite.

VIII. Summary.

The black lines of Armillaria mellea have been found in the tissues of a number of plants. Isolations were made from the lines and an account of the develop-/ment of Armillaria mellea in culture is given. Particular mention is made of the formation of sclerotium like bodies in the culture medium and of the develop-/ment of the rhizomorphs. Pure cultures of the fungus on wood blocks have produced black lines in the sub-/stratum similar to those occurring in nature.

A short review is given of the nature of the sclerotium and from a consideration of the formation of the black plates, or lines as they appear in section, the suggestion is made that they form the limit-/ing layer of sclerotium-like bodies, which are partly immersed in the substratum. The black lines in the wood are continued at the surface as the black rind of an effused mycelial mass named the black xylostroma. Thus/

Thus there is formed a body comparable to a sclerotium in that it is completely surrounded by a black rind of bladder hyphae and has a medulla of hyaline hyphae. It is, however, partly immersed in the wood substratum and, in consequence, contains a considerable proportion of host tissues. Further this body, probably as a result of its immersion in a hard woody substratum, has developed the power of apical growth resulting in the free portion of the body growing out into a number of slender, flattened strands below the bark of the tree. These strands are the Rhizomorpha subcorticalis of Persoon from which, eventually, the more cylindrical Rh. subterranea grow out through the cracks in the bark and into the soil. It is proposed to name the body in the wood the pseudosclerotium. This term includes the whole body, namely, the portion within the wood bound-/ed by the black line; the free black xylostroma and its extensions produced by apical growth, the Rh. sub-/corticalis and the Rh. subterranea.

A short account is given of the biology of Armillaria mellea with particular reference to the pseudosclerotium and its significance in the reproduct-/ion of the fungus.

The author is, again, greatly indebted to Dr. Malcolm Wilson/

Malcolm Wilson for his constant interest and valuable criticism. Thanks are also due to Miss I. Lauder-Thomson for the material of Armillaria mellea on Veronica propinqua and to Mrs. N.L. Alcock for the use of the photograph Plate I, fig.1.

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Explanation of Plates I - III.

All figures refer to *Armillaria mellea*.

Plate I.

Fig. 1. Part of the infected shrub of *Veronica propinqua* showing the rhizomorphs (marked by white paper) entering the roots. x 1/10.

Fig. 2. 6 Year old Douglas fir showing the white xylostroma below the bark. The black lines have been exposed by scraping away the black xylostroma which covered them. A rhizomorph (marked by white paper) can be seen entering the roots. x 1/2.

Fig. 3. Black circles in the wood of an elm exposed by removing the black xylostroma. Pieces of the black xylostroma can be seen still attached to the wood. x 1/8.

Fig. 4. Piece of elm wood showing the ridged and fan-shaped black xylostroma forming the slender, flattened strands (<u>Rhizomorpha subcorticalis</u>) x 1/3.

Fig. 5. Part of a root of <u>Veronica propinqua</u> showing the black lines in the wood. Nat. size.

Plate II.

Cultural characteristics of <u>Armillaria mellea</u>.

Fig. 1. Rhizomorphs formed on malt agar in artificial culture. Some have entered the medium where they have given rise to a halo of dark hyphae. The white dots are young branch initials. Description in text page 9. x 3/4.

Fig. 2. Reverse of two cultures on malt agar slopes showing the formation of a black sheet of bladder hyphae down through the medium to make contact with the glass. As a result the lower part of the slope has not dried out as much as the top portion. Description in text page 8. x 1.

Fig. 3. Block of pine wood inoculated with the Strawberry strain of <u>Armillaria mellea</u> split open to/

to show the black lines in the centre of the bl
Incubated for 12 months. x 1.

Fig. 4. Rhizomorph formed in culture growing up the surface of a pine block. Young white branches can be seen growing out from the rhizomorph. Description in text page 11. x 1.

Fig. 5. Typical culture on oat agar showing the rhizo-/morphs coming to the surface and the mycelium spreading out from them to join up with the original mycelial plate. Description in text page 5. x 2/3.

Fig. 6. Reverse of culture on malt agar slope showing a sclerotium-like body in the depths of the medium. The body, which consists of a black rind surrounding a white medulla, has develop-/ed no rind where it comes into contact with th glass. Rhizomorphs can be seen arising from the body. Description in text page 7. x 1.

Plate III.

Fig. 1. Transverse section through Hartig's material of the Armillaria black line in pine showing the packing of the bladder hyphae in the tracheides. Note the clear-cut nature of the line with no signs of the sucessive stages described by Hartig and Hiley. Unstained.

x 250.

Fig. 2. Longitudinal radial section of pine inoculated with the Strawberry strain of _Armillaria mellea_ Note the larger size of the bladder hyphae. Unstained. x 150.

Fig. 3. Longitudinal tangential section of the black line in pine wood showing the close packing of the bladder hyphae in the tracheides without any tendency to spread. Unstained. x 150.

Fig. 4. Transverse section of _Veronica_ root showing a black circle which represents a section through the pseudosclerotium close to its apex. Unstained. x 60.

Fig. 5. Longitudinal section through the tip of a young rhizomorph. The medulla is seen to consist of thin, hyaline, longitudinal hyphae which gradually change into the brown bladder cells which comprise the rind. Around the periphery and especially at the apex is a weft of thin mucilaginous hyphae which are difficult distinguish in this photograph. Unstained. x 80.

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Plate I

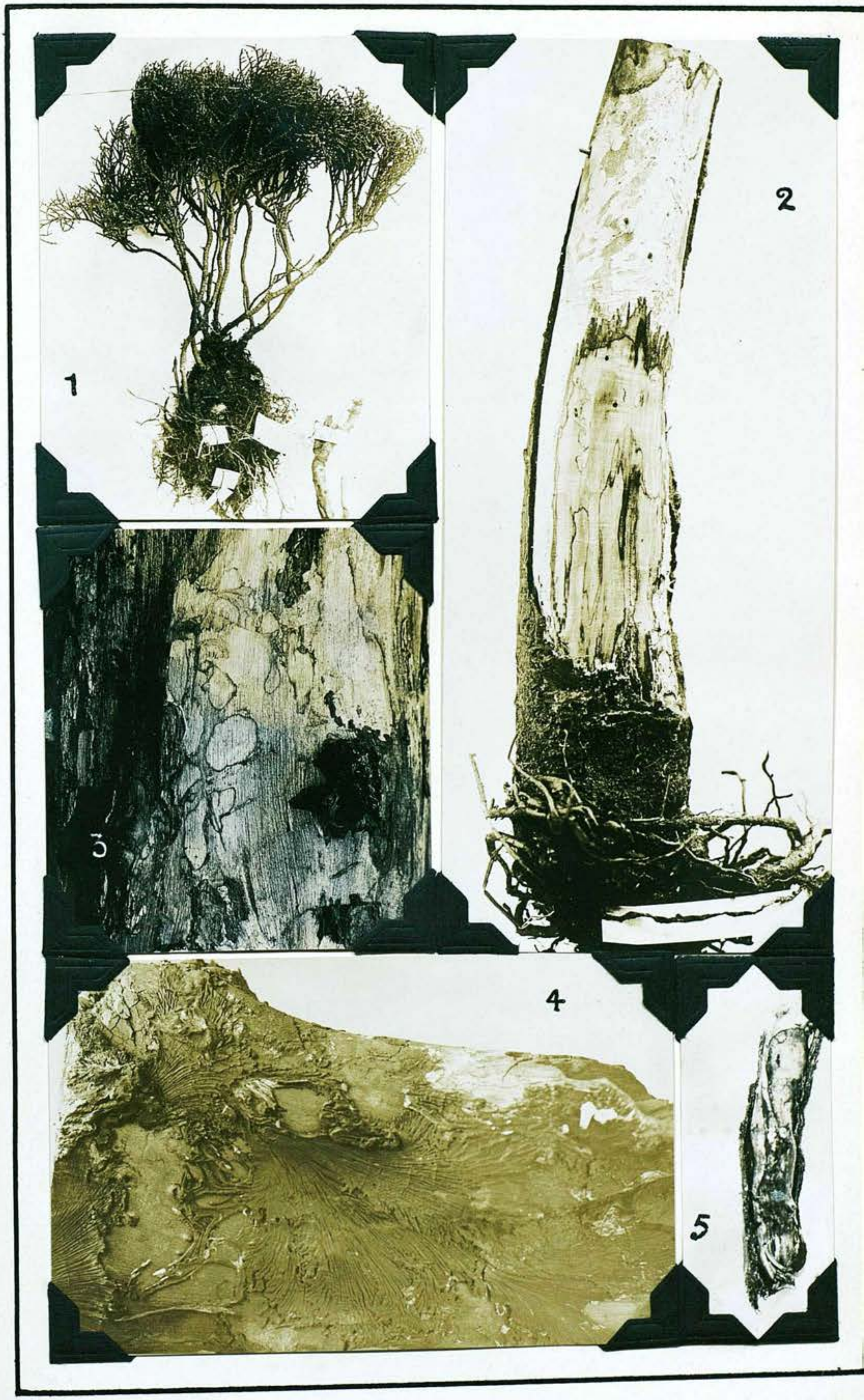


Plate II

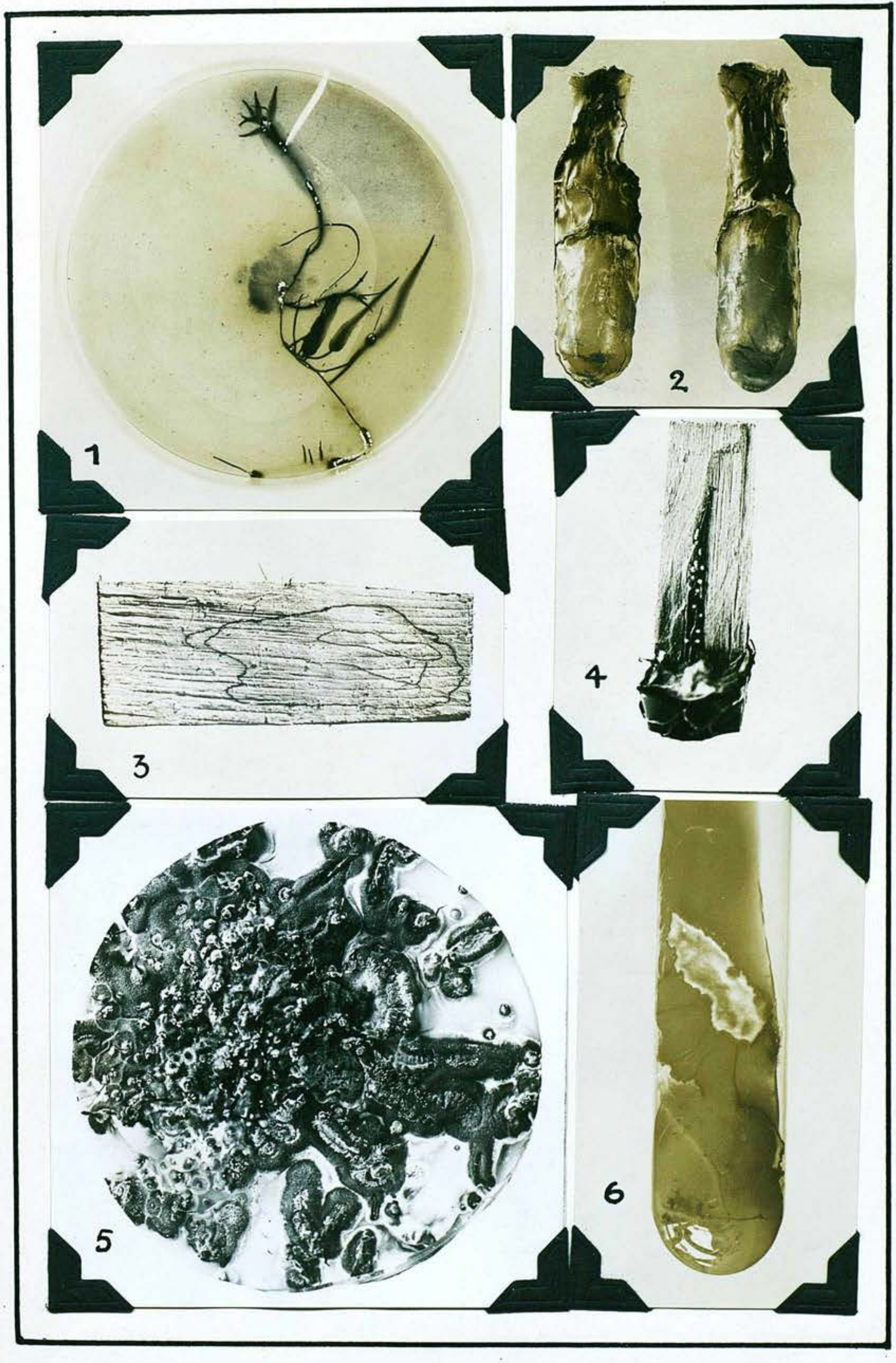
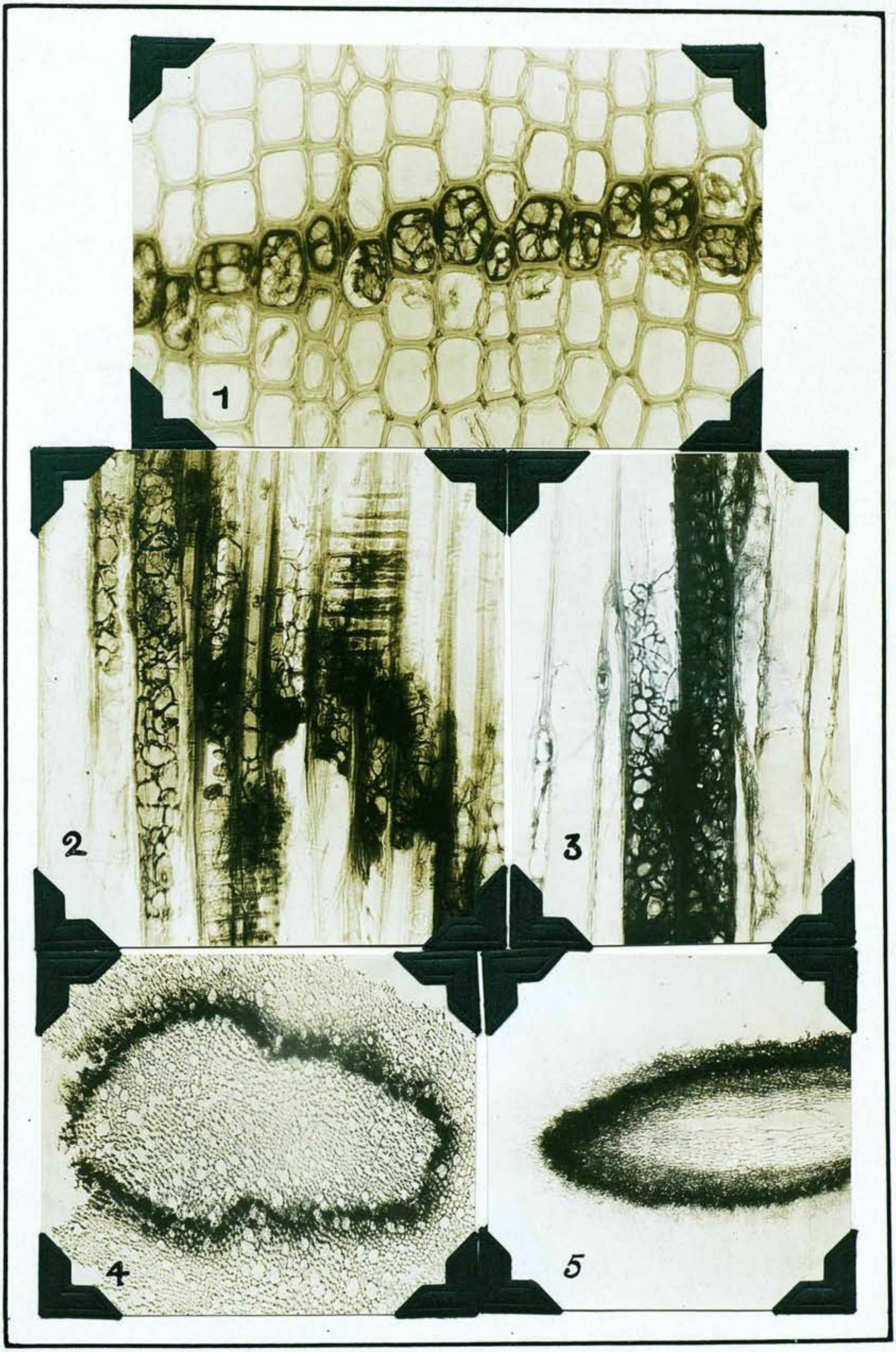


Plate III



CONCLUSION.

The two papers presented in this thesis deal chiefly with a single type of zone line, namely, the rind of a pseudosclerotium buried in the tissues of the host. Although this is the commonest kind of zone line, there are several other types of zone line about which very little is known. A brief outline of these lines has already been given on page 126 of the _Xylaria_ paper. One such line is formed by the deposition of "wound gum" as in the attack by _Fomes applanatus_ on beech, while another is formed by antagonistic mycelia on the same substratum as has been described by Weir (2) in the case of _F. pinicola_ and _F. fomentarius_ in birch. This latter phenomenon has also been described as being produced by strains of myxobacteria when colonised some little distance apart on agar (1). The writer has in his possession a zone line reputed to be formed between the rots of _Polyporus adustus_ and _F. igniarius_ but proof of this must await further investigation. Indeed, one of the difficulties of research on zone lines has been the necessity for working out the whole biology of the organisms concerned, as the zone lines, although often a matter for comment, have seldom been investigated. Many/

Many accounts of zone-producing fungi even omit to mention the presence of zone lines in the substratum. Thus before any decision as to the nature of the zone line can be made it is generally necessary to make a complete investigation of the fungus suspected of forming it.

This explanation of the zone line as the bounding layer of a pseudosclerotium in the substratum is one which may come to be applied to a large number of fungi. For example, it may be suggested that the transverse black lines on leaves attacked by Lophodermium pinastri are of such a nature, while it can readily be shown by inoculation that Sclerotinia fructigena will form black zones in apples. The writer believes that it will be possible to demonstrate all stages between the true sclerotium, the pseudosclerotium buried in the substratum and the case where part of the host, such as the fruit, forms in effect a sclerotium for the attacking fungus. Indeed, this study of zone lines is only beginning to uncover facts which may throw more light on the physiology of the fungi and their methods of reproduction.

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